TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

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Dedicated

to

my Family

&

Teachers

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Dr. Aakash Aggarwal

LIST OF ABBREVIATIONS

BMI	Body Mass Index
BSFS	Bristol Stool Form Scale
CFU	Colony Forming Units
FC	Fecal Calprotectin
FODMAP	Fermentable oligosaccharides, disaccharides, monosaccharides and polyols
GBT	Glucose Breath Test
GHBT	Glucose Hydrogen Breath Test
HAM-A	Hamilton Anxiety Rating Scale
HAM-D	Hamilton Depression Rating Scale
HBD2	Human Beta Defensin 2
HBT	Hydrogen Breath Test
HLA	Human Leucocytes Antigen
HPA axis	Hypothalamic Pituitary Adrenal Axis
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome with Constipation
IBS-D	Irritable Bowel Syndrome with Diarrhea
IBS-M	Mixed type Irritable Bowel Syndrome
IBS-QOL	Irritable Bowel Syndrome Quality of Life
IBS-SSS	Irritable Bowel Syndrome Severity Scoring System

LBT	Lactulose Breath Test
NCGS	Non-celiac gluten sensitivity
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PI-IBS	Post-Infectious Irritable Bowel Syndrome
SIBO	Small Intestinal Bacterial Overgrowth

ABSTRACT

Introduction: Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits. The pathophysiology of IBS remains uncertain. Role of inflammation, alterations in fecal flora, and bacterial overgrowth in the pathogenesis of IBS needs investigation. Further, the effect on these factors on severity of symptoms and quality of life in IBS patients also needs assessment.

Aim of Study: To study the correlation between small intestinal bacterial overgrowth (SIBO) and fecal calprotectin in patients with irritable bowel syndrome.

Materials & methods: This was a prospective observational study, which enrolled IBS patients (Rome IV). SIBO was diagnosed using GHBT. Fecal calprotectin was measured in each patient for evaluation of intestinal inflammation. Relationship of these factors with symptom score (IBS-SSS) and quality of life scores (IBS-QOL) was studied.

Results: Total 112 IBS patients (mean age 35.8 ± 11.9 years; male:female - 1.9:1) were included in the study. Mean duration of symptoms was 2.7 ± 2.5 years. Mean IBS-SSS score was 215.2 ± 66.6 and mean IBS-QOL score was 66.7 ± 15.0. SIBO was present in 32.1% of the IBS patients. Mean fecal calprotectin level was 41.6 ± 27.2 μg/g and it was raised (>50 μg/g) in 27.7 % of IBS patients. Out of 36 patients who had SIBO, fecal calprotectin was raised in 29 (80.6%) (p=0.001). Patients with SIBO or raised fecal calprotectin received Rifaximin treatment for 2 weeks by the primary treating physician. At 12 weeks follow-up, these patients reported significant improvement in IBS-SSS and IBS-QOL scores.

Conclusion: Significantly higher number of SIBO positive IBS patients had raised fecal calprotectin levels as compared to those with SIBO negative patients (p = 0.001). Mean IBS-SSS and IBS-QOL were significantly higher in those IBS patients who had SIBO or raised fecal calprotectin; and these scores improved after treatment.

Keywords: *Fecal calprotectin, IBS: Irritable Bowel Syndrome, SIBO: Small Intestinal Bacterial Overgrowth*

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INTRODUCTION

Irritable bowel syndrome is a functional gastrointestinal disorder that presents with symptoms of recurrent abdominal pain associated with changes in bowel habit without organic abnormalities. Prevalence of IBS ranges from 9% to 29.2% in the west and 3.5% to 14.2% in the east.[1,2,3] Patients experience abdominal pain and altered bowel habits, with either predominantly diarrhea (IBS-D), constipation (IBS-C), or both (IBS-M). There is no definitive biomarker for IBS, so IBS is mainly diagnosed clinically.

Various studies in IBS pathophysiology over the past decades have focused on dysmotility of the colon, visceral hypersensitivity, and the brain-gut interaction. Recently, other mechanisms have been actively studied, including inflammation, altered microbiota, dietary factors and enteroendocrine cells.[4] However, evidence regarding their role in IBS remains controversial. Recently, the definition of IBS has been questioned by increasing evidence of organic abnormalities in patients who fulfil the Rome criteria for IBS. Due to these new paradigms, IBS may no longer be classified as an absolute functional disorder.

The human GI tract is inhabited by a vast number of microbial populations including bacteria, fungi, and viruses. Bacteria contribute to the largest population of intestinal microbiota, consisting of 500 (using culture approaches) to 1,000 (by 16S rRNA gene sequencing) different bacterial species.[5] The small intestine comprises mainly of Gram positive and aerobic bacteria and the large intestine contains predominantly Gram negative and anaerobic bacteria. The stomach and proximal small bowel normally contain relatively fewer bacteria due to the presence of gastric acid and the effects of peristalsis. Lactobacilli, enterococci, gram-positive aerobes, or facultative anaerobes predominate in concentrations of 10^4 organisms/mL in the mid to distal jejunum. Concentration of the coliforms rarely exceeds 10^3 organisms/mL. On the other hand, majority of bacteria residing in the colon are strictly anaerobes (95% of total) followed by facultative

anaerobes and aerobes. Bacteroides, which are predominant organisms in the colon, are rarely found in the proximal small bowel.

Small intestinal bacterial overgrowth (SIBO) is a condition in which colonic bacteria are seen in excess in the small intestine. When present, this overabundance of organisms can result in IBS like symptoms like bloating, flatulence, abdominal discomfort, or chronic watery diarrhea and in extreme cases, malabsorption.

Although quantitative culture of the upper gut aspirate has traditionally been utilized as the gold standard for the diagnosis SIBO, its limitations include difficulty and invasiveness, cost, contamination by oropharyngeal flora, and inability to culture as high as 70% bacteria colonizing the gut. Moreover, distribution of bacterial overgrowth in intestine may be patchy and upper gut aspirate may not be able to detect bacterial overgrowth in distal gut.

Breath tests are popular, non-invasive and patient-friendly methods used increasingly for diagnosis of SIBO. Breath tests are based on the principle that metabolism of a test dose of carbohydrate substrate (e.g., lactulose, glucose) by the bacterial flora leads to the production of an analyte (hydrogen, methane), which is absorbed and ultimately excreted in the breath. Eighty percent of the gases like hydrogen and methane are eliminated with the flatus and the remaining twenty percent are absorbed and exhaled via lung, which can be measured in breath. Using hydrogen breath tests, prevalence of SIBO in IBS range from 6.2% to 46%.[6]

Glucose and lactulose are the most common sugar substrates used in hydrogen-based breath testing. Glucose is a monosaccharide that is absorbed primarily in the proximal small intestine, whereas lactulose is a synthetic nonabsorbable disaccharide that undergoes fermentation in the colon. Under normal physiologic conditions, glucose is almost entirely absorbed in the proximal small intestine. In the presence of bacterial overgrowth, however, glucose is fermented, releasing its gases, which are then absorbed into the bloodstream and expired by the lungs. Similarly, with lactulose, in the presence of excessive bacteria in the small intestine, lactulose undergoes fermentation earlier than

expected. Classically, using the lactulose breath test (LBT), the presence of a “double peak,” with the first peak due to hydrogen production by the bacterial population in the small intestine and the second peak due to the action of colonic microbes on lactulose, was felt to be representative of the presence of SIBO.

To diagnose SIBO, 75 g of glucose is given in 250 mL of water with breath samples collected every 15 to 20 minutes for a total of 120 to 180 minutes. An increase in hydrogen level by ≥20 parts per million (ppm) in breath sample over baseline and sustained over at least 2 time points within 90 minutes is considered consistent with SIBO.[7]

Role of inflammation has been emerging out to be one of the important etiological factors in pathogenesis of IBS. Immunohistological assessment in IBS patients have showed an increased number of activated immunocompetent cells, including T-lymphocytes, neutrophils, and mast cells in the mucosa of intestine, implying the role for the mucosal immune system in pathogenesis.[8] Mast cells are associated with wound healing, defence against pathogens, and hypersensitivity in GI mucosa. They degranulate to release inflammatory and immune mediators, which lead to the recruitment of other inflammatory cells into the GI mucosa. Several studies have showed that increased mast cells in IBS patients may correlate with certain symptoms of IBS, such as abdominal pain and bloating.[9] Another form of cells, that may play a pathological role in IBS are activated T-lymphocytes. T lymphocytes are involved in adaptive immunity and have numerous functions, such as the activation of B lymphocytes and macrophages and the destruction of infected host cells. In addition, enhanced expression of proinflammatory cytokines in peripheral blood mononuclear cells and serum may confer a predisposition to immune activation in patients with IBS.

Other than the inflammatory cells, pathogen related factors also play role in development of IBS. One group have reported increased levels of beta-defensin 2 peptides (HBD-2) in fecal fluid derived from IBS patients.[9] HBD-2 is an antimicrobial peptide recently implicated in the pathogenesis of inflammatory bowel disease. These results suggest an activation of the mucosal innate defence system towards a

proinflammatory response in IBS patients without macroscopic signs of inflammation.

Another evidence of role of inflammation in IBS is IBS-onset following an episode of gastroenteritis, called post-infectious IBS or PI-IBS. Inflammation along with immune cells play roles in the intestinal neuroendocrine system, which controls GI sensory-motor function. Serotonin secretion from enterochromaffin cells, which regulates the gut immune system, can be attenuated by the secretory products of immune cells.[10] Flagellin antibodies are observed more frequently in patients with PI-IBS, indicating that immune activation in response to luminal triggers play a role in the development of IBS. Flagellins are primary triggers of innate and adaptive immunity, thus driving pathogen-induced acute inflammation.[11] These observations suggest that inflammatory responses to infection, rather than the infective pathogen itself, are an important predisposition to the occurrence of IBS, particularly post-infectious IBS.

Recently, the evaluation of fecal levels of calprotectin, a neutrophil-selective protein, has been proposed as marker of subclinical inflammation in the gastrointestinal tract.[12] Calprotectin is a zinc and calcium binding protein that is derived mostly from neutrophils and monocytes. It can be detected in tissue samples, body fluids, and stools, making it a valuable marker of neutrophil activity. The use of the calprotectin level as a biomarker of intestinal inflammation could represent a noteworthy targeted therapeutic approach not only to rule out IBD, but also to identify potential IBS candidates for an anti-inflammatory treatment. Level of fecal calprotectin in patients with IBS have been found to be between 10-210 μg/g.[13]

Assessment of quantification of severity of symptoms and quality of life in patients of IBS is important to improve the treatment outcome. With a score based on standardized questionnaire, the patient can communicate and express to physician that how the illness has made an impact in his or her life. The physician can also assess level of functioning of patients and objectively track them to gauge response to

treatment. IBS-SSS score is used to measure severity of symptoms in IBS patients, which consists of 5 equally weighted questions about symptoms occurring in the past 10 days: the average intensity of abdominal pain, the number of days with any abdominal pain, the average severity of abdominal distension, dissatisfaction with bowel habits, and the degree to which bowel symptoms interfered with usual activities.[14] IBS-QOL score is used to measure quality of life, which is a 34-item self-report questionnaire with 8 subscales: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction and sexual relationship.[15]

A strong correlation can be observed between the severity of IBS and its comorbid psychiatric disorders. Various studies have reported prevalence of anxiety or depression in IBS patients from 30-40%, other have reported them to be as high as 80%.[16] The inflammatory hypothesis of mental health states that persistent immune activation and the resultant inflammatory mediators (e.g., by gut microbiota dysbiosis) may dysregulate the brain's stress systems, notably the hypothalamic-pituitary-adrenal (HPA) axis. As exemplified, gut microbiota dysbiosis can produce pro-inflammatory communication that impairs downregulation of HPA axis hormones involved in neural pathways of stress. Consistently, elevated levels of circulating cortisol and inflammatory markers have been associated with anxiety and depression.[17]

Data on correlation between SIBO and intestinal inflammation measured by fecal calprotectin levels is scarce and controversial. This study intended to assess the correlation between small intestinal bacterial overgrowth and fecal calprotectin in patients with irritable bowel syndrome. We also aimed to assess the correlation between SIBO and intestinal inflammation with symptom severity, quality of life, anxiety and depression in IBS patients.

AIMS AND OBJECTIVES

Primary aims:

- To study the frequency of small intestinal bacterial overgrowth (SIBO) in patients with irritable bowel syndrome (IBS).
- To study what proportion of IBS patients have evidence of intestinal inflammation (i.e., raised stool calprotectin).
- To study the co-relation between SIBO and fecal calprotectin in IBS patients

Secondary aim:

- To study the co-relation between quality of life and symptoms severity with SIBO and calprotectin in patients with IBS.

REVIEW OF LITERATURE

Irritable bowel syndrome is a functional bowel disorder characterised by chronic or recurrent abdominal pain associated with either relief or exacerbation by defecation, or a change in bowel habits. Patients experience abdominal pain and altered bowel habit, with either predominantly diarrhea, constipation, or both. There is no definitive investigation as no biomarker has been found, so IBS is diagnosed clinically. The earliest reports about IBS dates back to 19th and 20th century. At that time, diagnosis was only made by exclusion of malignant, inflammatory, or infectious disease after full investigations and extensive unsuccessful surgeries.[18] IBS remained frequently misdiagnosed and poorly understood late up to 1970s, with the problem of unsuccessful, or unnecessary, surgery continuing. Manning et al created the first set of formal criteria to standardize definition of IBS that allowed IBS to be positively diagnosed without the need for extensive investigations to exclude other diagnosis.[19] These criteria were developed through expert consensus to create the Rome consensus criteria, now in its fourth iteration.[20] The Rome consensus criteria also recommend that IBS should be a positive diagnosis but is more restrictive than the Manning criteria. Patients with IBS report symptoms of abdominal pain at least once per week (on average) in association with a change in stool frequency, a change in stool form, and/ or relief or worsening of abdominal pain linked to defecation. Accordingly, IBS is a heterogeneous syndrome and diagnosis relies on report of symptoms by patients.

EPIDEMIOLOGY

Prevalence of IBS ranges from 9% to 29.2% in the west and 3.5% to 14.2% in the east, based on Rome II/III criteria.[1,2,3] A population-based survey using census-adjusted Rome IV functional bowel disorders reported that prevalence of IBS in Western countries ranges from 4%-5%,[21] while in Asian countries it ranges between 1% and 10%.[22] In a meta-analysis that included eight international studies, the pooled prevalence of IBS was estimated to be 11 %, with wide variation by

geographic region.[23] The prevalence of IBS in India is estimated at 4.0%-7.9% and continues to be on the rise.[24] Ghoshal et al[25] did a multi-centric study in pan-India and found prevalence of IBS to be 4.2 %. Makharia et al[24] found it to be 4% in a north Indian population. Goyal et al[26] found prevalence of IBS to be 6.2% among college students in northern India.

PATHOPHYSIOLOGY

Traditionally, IBS has been conceptualized as a condition of visceral hypersensitivity (leading to abdominal pain or discomfort) and gastrointestinal motor disturbances (leading to diarrhea or constipation). The gastrointestinal motor disturbances identified, including changes in intestinal transit, do not easily explain mixed or alternating IBS. It has been suggested that these abnormalities are secondary to psychological factors rather than being of primary relevance. However, not all patients with IBS have significant psychological disturbances and referral bias may account for the psychological associations. Abnormalities in visceral hypersensitivity and gastrointestinal motor disturbances are also emerging. There is increasing evidence of organic disease in the gastrointestinal tract in subset of patients who fulfil the Rome criteria for IBS. Evidence for subtle inflammatory bowel disease, serotonin dysregulation, bacterial overgrowth and central dysregulation continue to accumulate. Furthermore, genetics may also contribute to IBS.

Gastrointestinal motility

Motor abnormalities of the gastrointestinal tract (GI) are detectable in patients with IBS. These include increased frequency and irregularity of luminal contractions, prolonged transit-time in constipation-predominant IBS, and an exaggerated motor response to cholecystokinin and meal ingestion in diarrhea-predominant IBS.[27]

Visceral hypersensitivity

Visceral hypersensitivity i.e., increased sensation in response to stimuli, is a common finding in IBS patients. Various studies have shown that in IBS patients, awareness and pain caused by balloon distention in the intestine are experienced at lower balloon volumes compared with controls, suggesting receptor hypersensitivity.[28] This possible increase

in sensitivity may be specific for visceral afferents, since it is reported that IBS patients have normal or even increased thresholds to somatic pain. About 50% of the IBS patients (mainly IBS-C) have a measurable increase in abdominal girth associated with bloating (sensation of abdominal fullness), which may not be related to the volume of intestinal gas. Patients who complain of bloating actually had volumes of gas in their gut similar to asymptomatic controls, but exhibit impaired transit of intestinal gas loads.[29]

Disturbances in Gut-Brain axis

IBS symptoms that do not relate to the gastrointestinal tract, most notably anxiety and depression, are highly prevalent in outpatient and community samples and these associations are not explained by health-care-seeking behaviour alone. Such observations have led many to conceptualise IBS as a primary disorder of brain–gut function, or even primary somatisation, with the brain leading to gut manifestations, fatigue, and other complaints.[30] In an independent study of IBS and psychiatric disorders, the use of structured interviews showed that 23% of patients with anxiety and 40% of patients with mood disorder developed these diagnoses after the onset of IBS.[31]

Altered Gut microbiota

Changes in the intestinal environment due to altered gastrointestinal motility provide favourable conditions for colonic bacteria to outnumber normal commensals of small intestine. An altered gut microbiota may implicate specific metabolic pathways or interactions between microbes and the immune and endocrine systems—opening new possible directions for therapy. A study done in Indian population by Shukla R et al[32] evaluated fecal microbiota in IBS patients and compared them with healthy controls, and found that fecal microbiota was significantly different among IBS than healthy controls. *P. aeruginosa* was more frequent and higher in number among IBS patients. Intestinal microbiota provides a link between the subclinical intestinal inflammation and symptoms of IBS. In a recent review by Pittayanon et al[33], IBS cohorts had a higher abundance of the bacterial phylum Proteobacteria, the families Lactobacillaceae and Enterobacteriaceae, as well as the genus

Bacteroides relative to controls. Participants with IBS also had lower levels of the genera Bifidobacterium and Faecalibacterium. Alpha diversity, which is a measure of the number of species in a community was also lower in IBS.

Intestinal inflammation, altered permeability & immune activation

Numerous studies, summarized in a systematic review, have shown that low-grade mucosal inflammation can be identified in some individuals with IBS, if strict criteria are applied.[34] This concept was first proposed almost 25 years ago, and although in most patients no cause can be found, there is a possibility that either a previous, or previously unrecognized, infection contributes to or is the cause of this mucosal inflammation.

Immunohistological investigation has revealed mucosal immune system activation characterized by alterations in particular immune cells and markers in some patients with IBS (those with diarrhea-predominant IBS and patients with presumed postinfectious IBS). Fecal calprotectin is a sensitive marker of intestinal inflammation due to wide variety of causes; it identifies patients with IBD (in whom levels may be used to predict impending relapse of disease) patients with NSAID enteropathy, and patients with colorectal carcinoma/adenoma, in all of which there is increased neutrophil recruitment to the inflamed or ulcerated intestinal mucosa. In functional bowel diseases mean fecal calprotectin level is generally found to be <50 μg/g of stools, that differentiates it from IBD in which mean level is >500 μg/g.[35] Irritable bowel syndrome, due to better understanding of its pathophysiology over time, is emerging as a disease with chronic sub-clinical inflammation at mucosal level. Although, level of fecal calprotectin in IBS may not be as high as seen in some of the above-mentioned organic diseases, it still might be higher than what is seen in healthy controls.

Michael J et al[36] compared fecal calprotectin level among IBS patients and healthy controls and found no significant difference between two groups. Carroccio et al[13] also studied fecal calprotectin in patients with chronic diarrhea and compared its level between IBS patients and

healthy controls. Fecal calprotectin was found to be elevated in IBS patients (Median=35, range 10-210 μg/g of stools) as compared to healthy controls (Median=20, range 10-40 μg/g of stools). Although, this difference was not statistically significant.

Burri E et al[37] found that fecal calprotectin level of 50 μg/g of faeces, discriminated organic from non-organic intestinal disease with 84.4% sensitivity and 94.5% specificity, in 405 symptomatic patients referred for colonoscopy. Also, there is evidence that calprotectin level can be indicated as a non-invasive test to identify persistent occult inflammation among IBD patients in clinical remission.

Using the above-mentioned cut-off value, Melchior et al[38] studied IBS patients to find and characterize patients with high fecal calprotectin. Mean fecal calprotectin in 93 IBS patients was found to be 90.5 ± 180 μg/g and a subgroup of 34 patients (36.6%) had levels higher than 50 μg/g. No patient with abnormal levels had diverticular disease or colorectal adenoma (confirmed by colonic biopsies) or was taking medications (mainly nonsteroidal anti-inflammatory drugs). Patients with high fecal calprotectin were followed for 1 year, but none of them developed IBD. This study underlines the idea that some proportion of IBS patients do have ongoing sub-clinical mucosal inflammation, marked by high fecal calprotectin. Shulman et al[39] showed a positive correlation between fecal calprotectin levels and IBS in children. He found that fecal calprotectin concentration was greater in children with FAP/IBS compared with control children (65.5 ± 75.4 μg/g stool vs 43.2 ± 39.4, respectively; $p < 0.01$). Fecal calprotectin concentration correlated with pain interference with activities. ($p < 0.05$)

FACTORS THAT CAN TRIGGER THE ONSET OF IBS

The development of IBS following infectious enteritis can be suspected clinically based upon a history of an acute diarrheal illness preceding the onset of IBS like symptoms. It can be associated with bacterial, protozoan, helminth and viral infections. The cause of bowel symptoms following acute infection is undetermined, although various theories have been proposed, which include malabsorption, increased

enteroendocrine cells and use of antibiotics during infection. The development of idiopathic bile acid malabsorption has been observed following enteric infections, which may be result in diarrhea-predominant IBS. An increase in serotonin-containing enteroendocrine cells and T lymphocytes, has been demonstrated following acute Campylobacter enteritis.[40] The increased serotonin levels lead to increased GI motility and visceral hypersensitivity.[41] One study suggested that increased numbers of enteroendocrine cells and depression were independent predictors of developing post-infectious IBS.[42] The use of antibiotics for GI or other infections was observed to be a risk factor for developing functional bowel symptoms.

Many IBS patients report worsening of symptoms after eating and perceive food intolerance to certain foods. One etiology of IBS suggests that symptoms may be related to impaired absorption of carbohydrates. This theory originated in studies of patients with inflammatory bowel disease (IBD), but has been proposed as well for IBS patients. The theory holds that fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs) in patients with IBS or IBD enter the distal small bowel and colon where they get fermented, leading to symptoms and increased intestinal permeability and possibly inflammation.[43] Fructose intolerance has been suggested as a possible form of carbohydrate malabsorption contributing to gastrointestinal (GI) symptoms such as flatus, pain, bloating, belching and altered bowel habits.

In a proportion of patients, gluten sensitivity causes functional bowel disorder like symptoms. One study suggested that in patients without villous atrophy, the presence of serum IgG antigliadin antibodies and expression of HLA-DQ2 (associated with celiac disease) may predict response to a gluten free diet in patients with diarrhea-predominant IBS.[44] A study in patients with irritable bowel syndrome with diarrhea without celiac disease found that dietary gluten altered small intestinal permeability and had a greater effect on bowel movement frequency in patients who were HLA-DQ2/8 positive compared with those who were HLA-DQ2/8 negative.[45]

FACTORS INCREASING RISK OF DEVELOPMENT OF IBS

IBS symptoms can be exacerbated by stress and are frequently associated with psychological co-morbidities. The most common comorbid psychiatric disorders seen include anxiety (panic and generalized anxiety disorder), somatoform disorders (hypochondriasis and somatization disorder), depression (including dysthymia) and phobic disorders. Psychological distress can also affect the somatic symptoms and its outcome; it lowers the threshold for pain and influences health care seeking behaviour. Research on risk factors such as smoking and alcohol consumption has shown mixed association.[46]

There also may be a background genetic predisposition. Some studies show a higher concordance rate for IBS in monozygotic twins compared with dizygotic twins, with concordance rates for IBS in monozygotic twins ranging from 2 to 22 % and rates in dizygotic twins ranging from 1 to 9%.[47] However, in a study of 5032 twins (888 monozygotic pairs and 982 dizygotic pairs) the concordance rate did not significantly differ between monozygotic and dizygotic twins (17 vs 16%).[48] In addition, one study found that having a parent with IBS was a greater independent predictor of IBS than having an affected twin, suggesting that the familial nature of IBS could be due to social learning and genetics.[49] Mutation or polymorphisms in the serotonin transporter gene can lead to development of IBS, as altered serotonin reuptake affects intestinal peristalsis.

A poor socioeconomic status during childhood has been associated with worse health care outcomes in adulthood. Another study found a significant correlation between childhood higher affluence and positivity to the Manning criteria.[50] One potential explanation for these conflicting outcomes is that higher socioeconomic status is associated with better access to health care or to a greater tendency to seek a physician's advice and diagnosis. Moreover, in higher social classes, behaviour leading to internalization of stress also may be promoted, and this component (rather than stress itself) may result in functional GI disorders.

CLINICAL FEATURES

IBS is characterized by the main features of chronic abdominal pain and altered bowel habits. Abdominal pain in IBS is described as a cramping sensation of variable intensity and periodic exacerbations. The location and character of the pain can vary widely. The severity of the pain can be mild to severe and is related to defecation. While in some patients abdominal pain is relieved with defecation, some patients report worsening of pain with defecation. Emotional stress and meals can also exacerbate the pain. IBS patients also commonly report abdominal bloating and increased gas production in the form of flatulence and/or belching. Symptoms of IBS also include diarrhea, constipation, alternating diarrhea and constipation, or normal bowel habits alternating with either diarrhea and/or constipation.

Altered bowel habits includes diarrhea, constipation or mixed symptoms. Diarrhea is commonly characterized as frequent loose stools of small to moderate volume. Bowel movements generally occur during waking hours, most often in the morning or after meals. Most bowel movements are preceded by lower abdominal cramping pain, urgency, and a sensation of incomplete evacuation or tenesmus. Constipation is defined as hard or sometimes pellet-shaped stools.

The **Bristol Stool Form Scale (BSFS)** is a diagnostic tool designed to classify the form of human faeces into seven categories.[51] The seven types of stool are:

Type 1: Separate hard lumps, like pellets

Type 2: Sausage-shaped but lumpy

Type 3: Like a sausage but with cracks on its surface

Type 4: Sausage or snake like, smooth and soft (average stool)

Type 5: Soft blobs with clear cut edges

Type 6: Fluffy pieces with ragged edges, a mushy stool

Type 7: Watery, no solid pieces, entirely liquid

Types 1 and 2 indicate constipation, with 3 and 4 being the ideal stools as they are easy to defecate while not containing excess liquid, 5 indicating lack of dietary fibre, and 6 and 7 indicate diarrhea.

Assessment of symptom severity (IBS-SSS)

Assessment of severity of symptoms is important for improved communication between patients and clinicians to monitor the effectiveness of therapy. Among the different severity scores published in the literature, the score developed by Francis et al[14], called IBS – symptom severity score is easy to use and is reproducible. It is widely accepted score for measuring the overall severity of IBS symptoms. It is valuable in clinical settings to monitor patient progress as well as for research purposes, to recruit homogenous group of IBS patients. It contains five 100-point scales based on the symptoms occurring in the past 10 days, that assess the severity of abdominal pain, the frequency of abdominal pain, the severity of abdominal distention, dissatisfaction with bowel habits, and interference with quality of life. All 5 components contribute to the score equally giving a range of 0–500, with a higher score indicating greater symptom severity. Patients can be categorized as having mild IBS (75-175), moderate IBS (175-300), or severe IBS (>300). A decrease of 50 points is associated with a clinically meaningful improvement. Coffin et al also found a positive correlation between symptom severity and quality of life.[52]

Assessment of quality of life (IBS-QOL)

It is estimated that more than a half of IBS patients suffer from psychological disorders, which impede treatment and decrease quality of life. In IBS there is no specific biological disease marker, the most important criterion to estimate the level of recovery is quality of life assessment. Even if measures of disease activity, symptoms and duration are available based on imaging, laboratory or endoscopic data, they do not always correspond with well-being in chronic gastrointestinal diseases.[53] The most well-known, popular and validated specific measure of health related QOL in IBS patients is the Irritable Bowel Syndrome-Quality of Life (IBS-QOL) questionnaire developed by D.L. Patrick and D.A. Drossman in 1998.[54] IBS-QOL is a self-report quality of life measure, that has undergone many iterations since its inception. Presently used IBS-QOL is a 34-item questionnaire, which includes domains such as dysphoria, interference with activity, body image,

health worry, food avoidance, social relation, and sexual and relationship issues. Each of these provides a subscale score and summates to give an overall score. A five-point Likert scale is used to assess how much the statement described the feelings of the respondent: not at all, slightly, moderately, quite a bit, and extremely or a great deal. The IBS-QOL is then transformed into a 0–100 scale, where 0 represents minimum QOL and 100 represents maximum QOL. It has shown a high internal consistency, reliability and reproducibility.[55]

IBS-QOL has also been shown to be responsive to treatment changes over time. A reduction of 14 points in IBS-QOL translates to a clinically meaningful improvement. This has been validated by Drossman et al in a multicentric trial on females with IBS who were undergoing treatment.[56] The study showed significant correlation between change in scores on the IBS-QOL and other measures of treatment effect. Similar study also demonstrated responsiveness of the instrument, with the IBS-QOL being able to differentiate responders from non-responders. More recently, in a randomized double-blinded clinical trial assessing the efficacy of lubiprostone in constipation-predominant IBS patients, the IBS-QOL demonstrated a clinically meaningful difference in the domains of social reaction, food avoidance, health worry, body image and dysphoria.[57] Kopczyńska et al also found significant differences in IBS-QOL between IBS and control group, particularly in overall quality of life, where mean IBS-QOL score was 61.62 ± 19.88 in IBS patients compared to 98.30 ± 4.11 in controls ($p < 0.0001$).[58]

Psychological co-morbidities (anxiety & depression) in IBS

Depression and anxiety disorders have been found in 38–100% of IBS patients.[59] Around 30% of people who experience the symptoms of IBS will consult physicians for their IBS symptoms. These people do not have different or severe abdominal symptoms as compared to those who do not consult, but they do have higher level of anxiety and lower quality of life.[60] Although complex interactions between biological, social and psychological factors likely contribute to the pathophysiology of these

conditions, an improved understanding is essential for developing therapeutic interventions. Psychosocial factor can act as trigger for the onset of IBS, and this has been studied extensively. Blaga et al studied about the psychosocial determinants of IBS and reported a significant increase in stressor score just before progression from IBS non-patient to IBS patient.[61] Major life traumas (e.g., disruption of a close relationship, a marital separation, a family member leaving home, or break-up of a serious girlfriend/boyfriend relationship) were frequently reported 38 weeks prior to onset of IBS symptoms.

There are questionnaire-based scores to evaluate anxiety and depression in IBS patients. The **HAM-A** was one of the first rating scales developed to measure the severity of anxiety symptoms, and is still widely used today in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both somatic anxiety (physical complaints related to anxiety) and psychic anxiety (mental agitation and psychological distress). Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0–56, where <17 indicates mild severity, 18–24 mild to moderate severity and 25–30 moderate to severe.[62] The **HDRS (also known as the Ham-D)** is the widely used clinician-administered depression assessment scale. The original version contains 17 items (HDRS17) related to symptoms of depression experienced over the past week. A later 21-item version (HDRS21) was developed that included 4 items intended to subtype the depression. A score of 0–7 is accepted to be within the normal range (or in clinical remission), while a score of 20 or higher (indicating at least moderate severity) is usually required for enrolment into a clinical trial.[63]

Various studies have shown that as many as 30-40% of patient with IBS have co-morbid depression or anxiety disorder.[64,65] Tangen et al studied the prevalence of mental disorders in clinical gastrointestinal populations in Asia and found it to between 60 to 85%.[66] Banerjee el al studied the Indian population and found the prevalence of depression to be 84% in IBS cohorts, and the prevalence of a comorbid anxiety disorder at 44%.[67] Liu et al assessed the gut microbiota in IBS patients and

suggested that IBS-D and depression cohorts may have similar bacterial imbalance, giving a common factor between IBS particularly diarrhea predominant and depression.[68] Kabra et al studied the presence of anxiety disorder and depression by using the Hamilton Anxiety rating scale and Hamilton Depression rating scale, respectively.[16] He found that the prevalence of depression and anxiety disorder in IBS was 37.1% and 31.4% respectively. IBS patients have been shown to have an overall higher degree of psychological symptoms in general and anxiety symptoms in particular. In one study the mean scores on SCL90 (Symptom Checking List questionnaire-90) for depression and anxiety, in all three IBS subtypes were higher than in healthy controls.[69]

DIAGNOSIS OF IBS

Irritable bowel syndrome should be suspected in patients with chronic abdominal pain and altered bowel habits (constipation and/or diarrhea). In the absence of a biologic disease marker, many symptom-based criteria have been proposed for the diagnosis of IBS. The most widely used among them are the Rome IV criteria. According to the Rome IV criteria, IBS is defined as recurrent abdominal pain, on average, at least one day per week in the last three months, associated with two or more of the following criteria:[70]

- Related to defecation
- Associated with a change in stool frequency
- Associated with a change in stool form (appearance)

IBS can be further classified into the following sub-types:

- IBS-C More than one-fourth (25%) of bowel movements with Bristol Stool Scale Types 1–2 and less than one-fourth (25%) with Types 6–7.
- IBS-D More than one-fourth (25%) of bowel movements with Bristol Stool Scale Types 6–7 and less than one-fourth (25%) with Types 1–2.
- IBS-M More than one-fourth (25%) of bowel movements with Bristol Stool Scale Types 1–2 and more than one-fourth (25%) with Types 6–7.

There is no definitive diagnostic laboratory test for IBS. The purpose of laboratory testing is primarily to exclude an alternative diagnosis. Along with CBC, fecal calprotectin or fecal lactoferrin, stool testing for giardia (antigen detection or nucleic acid amplification assay), serologic testing for celiac disease and CRP levels can be done. Other investigations include age-appropriate colorectal cancer screening, abdominal radiograph to assess for stool accumulation and determine the severity in IBS-C.

Additional testing can be done depending upon the presence of alarm features, which include, age of onset after age 50 years, rectal bleeding or melena, nocturnal diarrhea, progressive abdominal pain, unexplained weight loss, laboratory abnormalities (iron deficiency anaemia, elevated C-reactive protein or fecal calprotectin/lactoferrin) and family history of IBD or colorectal cancer. Although the presence of alarm features may identify patients who are more likely to have an organic disease, but most patients will ultimately have a negative evaluation.

Course of IBS

Symptoms experienced by patients with IBS fluctuate over time, some patients experience resolution of symptoms, others develop new ones. In patients who do report IBS symptom resolution, 45% will subsequently experience other functional gastrointestinal symptoms.[71] Up to two-thirds of IBS patients experience functional dyspepsia, the prevalence of which in patients with IBS is up to seven times higher than in controls.[72]

TREATMENT OF IBS

The initial step towards management of IBS is **patient education** and reassurance about the disease and its course. There should be a good clinician-patient relationship to validate the patient's symptoms and the clinician must establish realistic expectations with consistent limits and should involve the patient in treatment decisions. Further, it has been found that strict **low FODMAP diet** for short-term and modified low FODMAP diet for long term in IBS-D patients leads to significant improvement in symptoms and quality of life.[73] Gluten has been demonstrated to alter bowel barrier functions in patients with IBS-D, so gluten restriction can be tried. Nonceliac gluten sensitivity (NCGS) has

also been hypothesized as an underlying mechanism for symptom generation in patients with IBS. Physical activity should be advised in patients with IBS given a potential benefit with regard to IBS symptoms and the general health benefits of exercise. Patients with mild IBS symptoms usually respond to initial non-pharmacological therapies.

Patients with moderate to severe disease do require adjunctive **pharmacological** therapy. Medications are given according to the predominant symptoms. **Constipation** predominant IBS patients can be given a trial of soluble fibres, psyllium husk and laxatives. Drugs available are lubiprostone, which is a locally acting chloride channel activator that enhances chloride-rich intestinal fluid secretion; linaclotide and plecanatide, which are guanylate cyclase agonists that stimulates intestinal fluid secretion and transit and tegaserod & prucalopride which are agonists of the 5-hydroxytryptamine-4 (5-HT4) receptor, stimulate the release of neurotransmitters and increase colonic motility. Drugs available for **diarrhea** predominant IBS patients are loperamide & eluxadoline, which are opioid agonists and alosetron, which is a 5-hydroxytryptamine-3 receptor (5HT-3) antagonist.

Apart from altered bowel habits, IBS patients also require management of bloating and spasmodic abdominal pain. **Antispasmodics** like dicyclomine and hyoscyamine can be administered on an as-per-need basis or in anticipation of stressors with known exacerbating effects. Antispasmodics provide short-term relief in abdominal pain in IBS patients, but their long-term efficacy has not been established. In a meta-analysis of five randomized trials, rifaximin was more efficacious than placebo for global IBS symptom improvement and was significantly more likely to be associated with decreased bloating as compared with placebo.[74] Schoepfer et al found that rifaximin treatment alleviated symptoms in patients with IBS who were LBT positive and this improvement was observed for a period of 3 months after 2 weeks of treatment with rifaximin.[75] Although the magnitude of benefit of probiotics and the most effective species and strain are uncertain, they have been associated with an improvement in symptoms.

IBS patients also have **non-gastrointestinal symptoms** like depression, for which anti-depressants are required.[76] Drugs like tricyclic antidepressants also have analgesic properties. A meta-analysis which included 12 randomized trials of antidepressants in adult IBS patients found that antidepressants were significantly more effective as compared with placebo in improving global IBS symptoms.[77]

SMALL INTESTINAL BACTERIAL OVERGROWTH

Small intestinal bacterial overgrowth is a condition in which the colonic bacteria are seen in excess in the small intestine. When present, this overabundance of organisms can result in IBS like symptoms like bloating, flatulence, abdominal discomfort, or chronic watery diarrhea and in extreme cases, malabsorption.

The origin of the term small intestinal bacterial overgrowth dates to literature exploring causes of malabsorption in patients with surgically created blind loops, intestinal fistula, diverticulosis of jejunum and intestinal pseudo-obstruction. In various studies, the ability of a Gram-negative flora of presumed colonic origin to metabolize amino acids, deconjugate bile acids, consume vitamin B12, synthesize folic acid and injure the small intestinal mucosa was demonstrated and a scientific basis for the malabsorption syndrome was provided.[78] Coliforms and Escherichia coli emerged as prime suspects. Clinical improvement or resolution of symptoms with antibiotics clinched the diagnosis.

From the initial studies the criterion of $>10^5$ CFU (colony forming units) per ml of jejunal fluid emerged as the diagnostic standard for SIBO.[79] The application of jejunal aspiration was limited by its invasive nature, problems with contamination and challenges in bacterial culture. The modern era of SIBO was ushered in by the advent of hydrogen breath tests. Initially employed in the diagnosis of carbohydrate malabsorption, hydrogen breath tests were soon applied to measure oro-caecal transit and detect bacterial overgrowth. Due to their simplicity and acceptability, glucose and lactulose breath tests came to dominate the field; they are the basis for virtually all the recent literature on SIBO.

Breath tests are based on the ability of small bowel bacteria to metabolize ingested lactulose or glucose into carbon dioxide and hydrogen and/or methane gas, which can then be captured using a breath analyser. The Rome consensus group recommends 50 g of glucose in 250 mL of water with breath samples collected every 15 to 20 minutes for a total of 120 to 180 minutes, whereas the North American consensus group recommends use of 75 g of glucose in 1 cup of water and a total testing period of 120 minutes. An increase in hydrogen level by ≥ 20 parts per million (ppm) in breath sample over baseline and sustained over at least 2 time points within 90 minutes is considered consistent with SIBO.[80]

Relationship between SIBO and IBS

IBS is manifested by abdominal pain and/or discomfort, irregular stool form and frequency. Bloating is reported by up to 80% of IBS patients and often is exacerbated by food.[81] Increased hydrogen production after the administration of fermentable substrate has been shown in IBS subjects compared with healthy controls and total excretion of hydrogen also may be increased in IBS. It has been suggested that the common explanation for increased hydrogen production, and symptoms such as bloating in IBS patients, is colonization of the proximal small bowel by fermenting bacteria, as occurs in SIBO. The concept of a possible association of SIBO with IBS has some rationale as many symptoms of these two conditions are similar, and that treatment of SIBO with non-absorbable antibiotics has shown improvement of symptoms in IBS patients. Studies have also suggested that while SIBO is associated with IBS with diarrhea, methane-positive breath tests are linked to constipation-predominant IBS.[82]

There is ample literature both from western world as well from India, in which relationship between IBS and SIBO was studied. Lapascu et al[83] studied SIBO in 65 patients with IBS, and compared them with 102 healthy controls. All subjects underwent glucose breath test, which was found to be positive in 31% of IBS patients as compared to 4% in the control group, the difference between groups was statistically significant. In a retrospective study, done by Cuoco et al[84] prevalence of SIBO was

found to be 45.8 % in IBS patients. Majevski et al[85] found that 46% IBS patients had SIBO. 73% of these were IBS-diarrhea dominant (IBS-D), 13% were constipation dominant (IBS-C) and 14% were IBS with alternating bowel pattern (IBS-M). In a study done by Lombardo et al[86] SIBO was detected in 24.5% of patients with IBS, and 6% of healthy control subjects; and the difference was statistically significant. In another study, done by Moraru et al[87] concluded that SIBO was present in 31.7% patients with IBS and in 6.6% healthy volunteers ($p < 0.0001$). Diarrhea and mixed symptoms were found to be predictive for SIBO. Abbasi et al[88] compared 107 IBS patients with similar number of healthy individuals, for the presence of SIBO. A peak of H2 values >20 ppm above the basal value after glucose ingestion was considered suggestive of SIBO. 37.4% of the IBS patients compared with 12.1% of healthy individuals had SIBO, ($p < 0.001$). Chu et al[89] studied data from 94 consecutive patients who met the Rome III criteria for IBS and 13 healthy volunteers. Participants also went concurrent 99mTc scintigraphy. The prevalence of SIBO in patients with IBS was higher than that in healthy controls (39% versus 8%.; $p = 0.026$). Wu et al[90] studied 60 IBS-D patients that fulfilled Rome IV criteria and 60 healthy controls, for the presence of SIBO using lactose breath. The prevalence of SIBO in IBS-D patients was higher than that in healthy controls (51.7% vs. 16.7%, p .001).

Rana et al[91] enrolled 225 consecutive patients of irritable bowel syndrome according to the Rome II criteria. Small intestinal bacterial overgrowth was estimated by using glucose hydrogen breath test. The prevalence of small intestinal bacterial overgrowth was 11.1% in patients with irritable bowel syndrome, which was significantly higher than apparently healthy controls. Sachdeva et al[92] included 59 consecutive patients of IBS (diagnosis based on Rome III criteria) & 37 healthy controls and SIBO was found to be more frequent in patients with IBS than HC. ($p = 0.008$). One study evaluated the difference between Lactulose and Glucose based Hydrogen Breath tests. Rana et al[93] enrolled 175 diarrhea-predominant IBS patients and 150 apparently healthy controls. SIBO was found to be positive in 34.3% patients by LBT

and in 6.2% patients by GBT. In controls, SIBO was positive in 30% by LBT and in 0.66% by GBT. Positive LBT was not significantly different in patients and controls; while using GBT, SIBO was significantly higher ($p < 0.01$) in patients as compared to controls. It was concluded that GBT was a better test to differentiate SIBO in IBS patients from controls. Role of SIBO in development of IBS had also been confirmed by the evidence of response in IBS symptoms, after therapeutic trial of rifaximin.[94]

Although abundant studies proved a positive corelation between IBS and SIBO, others failed to find such association.[95] The abnormal breath tests in patients with IBS may have been due to more rapid oro-caecal transit, rather than SIBO.[96] In addition, an improvement of symptoms with antibiotics in IBS patients may be due to an improvement in intestinal motility or a change in colonic flora rather than SIBO.[97] One study using cultures of jejunal aspirates from 162 IBS patients and 26 controls found SIBO ($>10^5$ CFU/mL) in 4 percent of both patients and controls. Although a mild increase in bacterial counts was more common in IBS patients (42 versus 12 %), this was not related to small intestinal motility and was not associated with symptoms. So far, the studies done for SIBO in IBS enrolled patients based on ROME II or ROME III criteria. Only few studies used Rome IV criteria to analyse SIBO in IBS patients.

FECAL CALPROTECTIN – A marker for assessment of intestinal inflammation:

Calprotectin is a calcium binding protein accounting for 60% of the cytosolic protein content of neutrophils. Accumulation of neutrophils at the site of inflammation in the gastrointestinal tract results in the release of calprotectin into faeces where it is stable and resistant to bacterial degradation. It identifies patients with IBD (in whom levels may be used to predict impending relapse of disease), patients with NSAID enteropathy, and patients with colorectal carcinoma/adenoma, in all of which there is increased neutrophil recruitment to the inflamed or ulcerated intestinal mucosa. Low level of inflammation has been reported in IBS, that can be detected using fecal calprotectin. The potential strength of fecal calprotectin is that it is a direct measure of

mucosal inflammatory activity that may be detected at a level insufficient to cause an increase in ESR and CRP, and levels in the stool seem to be unaffected by a variety of non-intestinal conditions, which may result in an elevation of the systemic inflammatory markers.

Association between fecal calprotectin, SIBO and IBS

There is scarce literature on measurement of fecal calprotectin in SIBO and its comparison with healthy controls. In IBS, there is change in gut microbiota that leads to development of SIBO and the presence of such bacteria in higher-than-normal concentration may be related with chronic sub-clinical inflammation of intestinal mucosa. Together these two factors can produce symptoms in IBS. Initial studies to evaluate relationship between fecal calprotectin and SIBO were not fruitful. For example, an analysis done by Montalto et al[98] in 2008, did not find a significant difference in level of fecal calprotectin among SIBO and control group. Further attempts also provided mixed results. While some failed to detect elevated levels in SIBO, studies on individuals with scleroderma[99] and Crohn's disease[100] suggested that elevated levels were a valuable indicator of the presence of SIBO.

David et al[101] evaluated IBS patients for SIBO, using GBT. Those who were positive for GBT were further tested for intestinal inflammation with a fecal test for calprotectin. It was found that a positive test for intestinal inflammation was significantly more frequent in those IBS patients who had SIBO ($p<0.05$).

Thus, it can be concluded that IBS, a functional bowel disease, has multi-factorial etiopathogenesis. There is no single cause that can be implicated in etiology, also no single mechanism can explain the pathophysiology of onset and symptom production. Research is needed to evaluate the association of SIBO and intestinal inflammation in IBS. Further assessment is also needed to study the effects of SIBO and fecal calprotectin on severity of symptoms, quality of life, anxiety and depression in IBS patients.

The present study aimed to assess the prevalence of SIBO and intestinal inflammation in IBS patients and their correlation. Further we aimed to

evaluate the effect of SIBO and intestinal inflammation on severity of symptoms, quality of life, anxiety and depression in IBS patients.

MATERIAL AND METHODS

METHOD OF COLLECTION OF DATA

Inclusion criteria – The study recruited 112 consecutive patients of IBS, who visited Gastroenterology OPD in DMC, Ludhiana, fulfilling the following criteria:

1. Age 18 to 60 years.
2. Fulfilling the diagnostic ROME IV criteria for IBS.

According to the Rome IV criteria, IBS is defined as recurrent abdominal pain, on average, at least one day per week in the last three months, associated with two or more of the following criteria[20]:

- Related to defecation
- Associated with a change in stool frequency
- Associated with a change in stool form (appearance)

Exclusion criteria – Following patients will be excluded from our study:

1. Active use of narcotics, alcohol, opioids or cigarette smoking.
2. Use of probiotics or antibiotics during the last 4 weeks.
3. Age of symptom onset > 50 years.
4. Rectal bleeding or malena.
5. Nocturnal diarrhea.
6. Progressive abdominal pain.
7. Unexplained weight loss.
8. Family history of IBD or colorectal cancer.
9. Laboratory abnormalities indicating an organic cause.
10. Iron deficiency anaemia.

After informed consent, patients were enrolled in the study. Complete medical history, physical examination, and routine lab investigations were noted. Results of the baseline hydrogen breath test (HBT), if done previously was noted. If baseline HBT results were not available, patients were advised to get it done, as it was required to rule out small intestinal bacterial overgrowth in IBS. Similarly, patients were advised to get fecal calprotectin if not done previously to rule out intestinal

inflammation. Further, the patients were classified into two groups, positive and negative, based on the breath test.

Positive Breath Test – Group A

Negative Breath Test – Group B

Both groups were compared for the association of intestinal inflammation with small intestinal bacterial overgrowth.

The IBS symptom severity scale (IBS-SSS), IBS quality of life scale (IBS-QOL), and psychological questionnaires (HAM-A and HAM-D) were administered to all patients and compared between both the groups.

Characteristics of the IBS patients were compared between breath test positive and negative group, as well as according to fecal calprotectin level.

Patients were followed up after 8-12 weeks to assess change in symptom severity and quality of life. Treatment given to the patient by his/her primary physician during this period was noted.

Questionnaires –

The **Irritable Bowel Severity Scoring System (IBS-SS)** is a validated scale for measuring the overall severity of IBS symptoms. It consists of 5 equally weighted questions about symptoms occurring in the past 10 days: the average intensity of abdominal pain, the number of days with any abdominal pain, the average severity of abdominal distension, dissatisfaction with bowel habits, and the degree to which bowel symptoms interfered with usual activities. Responses to all except the pain frequency question are on a 0-100 numeric scale (“none” to “worst ever”), and the number of days of pain in the past 10 days will be multiplied by 10 to arrive at a 0-100 score for this item. The scores on these 5 questions will be added to arrive a total score of 0-500.[14]

The **Irritable Bowel Syndrome Quality of Life Scale (IBS-QOL)** is a validated, disease specific scale to measure the impact of IBS symptoms on quality of life. It is a 34-item self-report questionnaire with 8 subscales: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction and sexual relationship. Subjects will be asked to answer questions about their feelings over the past 30 days on an ordinal scale (1-“not at all” to 5-“a great deal”).[15]

The **HAM-A** was one of the first rating scales developed to measure the severity of anxiety symptoms and is still widely used today in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Although the HAM-A remains widely used as an outcome measure in clinical trials, it has been criticized for its sometimes poor ability to discriminate between anxiolytic and antidepressant effects, and somatic anxiety versus somatic side effects. The HAM-A does not provide any standardized probe questions. Despite this, the reported levels of inter-operator reliability for the scale appear to be acceptable.[62]

The **HDRS (also known as the Ham-D)** is the most widely used clinician-administered depression assessment scale. The original version contains 17 items (HDRS17) pertaining to symptoms of depression experienced over the past week. Although the scale was designed for completion after an unstructured clinical interview, there are now semi-structured interview guides available. The HDRS was originally developed for hospital inpatients, thus the emphasis on melancholic and physical symptoms of depression. A later 21-item version (HDRS21) included 4 items intended to subtype the depression, but which are sometimes, incorrectly, used to rate the severity. A limitation of the HDRS is that atypical symptoms of depression (e.g., hypersomnia, hyperphagia) are not assessed.[63]

Breath test –

Breath analysis was done using GastroCH_4ECK® Gastrolyzer.

Principle - Hydrogen (H2) and Methane (CH4) are generated in the intestinal lumen by bacterial action on carbohydrates in the large or small intestine. Once the resultant H2 and CH4 are diffused into the bloodstream and then to the alveoli, it can be detected in expiratory air. There exists a correlation between intestinal lumen H2 and CH4 production and H2 and CH4 excretion in expiratory air. Accurate measurement of H2 and CH4 in parts per million (ppm) in expiratory air reveals abnormal breakdown and/or malabsorption of carbohydrates.

The GastroCH4ECK is a simple to operate device with options for both real time breath test and a bag sample test which allows patient's breath to be collected in breath bags and analyzed on the device. It also implements a normalizing factor for correcting errors in readings that could have occurred from sample contamination or incorrect breath sample collection. It incorporates single patient use mouthpieces and breath bags for cross contamination control.

Preparation for breath testing –

- No antibiotic, probiotic or bowel preparation (Peglac powder) 2 weeks prior to study.
- No laxatives, fibres or stool softeners, e.g., milk of magnesia, lactulose, isabghol, 1 week prior to study.
- On day before study, milk, juice, soft drink, soda, butter ghee, onion, garlic, cabbage, beans, pickle, pasta shall be avoided.
- Patients were instructed to skip morning medications and bring those medications along with them. They would have 12 hours fasting, but sips of water were allowed.
- On the day of study, chewing gum, smoking, or breath mints should be avoided.
- Patients were advised to brush teeth 2 hours prior to study.

Method – It is a simple, non-invasive test. After a short period of fasting i.e., 8-10 hours, a baseline end-expiratory breath sample was collected in breath bag and analyzed. Then, 75 g glucose was mixed with water and given orally. Subsequent breath samples are collected in breath bag and analyzed at every 15 minutes till 1 hour. Then, at the interval of 30 minutes, for the next 1 hour. Total, 6 samples were collected and analyzed over a period of 2 hours.

Interpretation - The glucose/lactulose breath test is diagnostic of SIBO if any one of the following criteria are met:

- An absolute increase in hydrogen by ≥20 ppm above baseline within 90 minutes.
- A methane level ≥10 ppm

Fecal Calprotectin-

Fecal calprotectin was measured using fluoro-enzyme-immunoassay technique. EliA^{tm} Calprotectin kit was used on the instrument Phadia 100.

Principle - The EliA^{tm} Calprotectin Wells were coated with monoclonal antibodies to calprotectin. If present in the patient's specimen, calprotectin would bind to the coated antibodies. After washing away non-bound components, enzyme-labeled antibodies against human calprotectin (EliA^{tm} Calprotectin Conjugate) would be added to form a calprotectin-conjugate complex. After incubation, non-bound conjugate were washed away and the bound complex was incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture was measured. The higher the response value, the more calprotectin was present in the specimen. To evaluate test results, the response for patient samples were compared directly to the response for calibrators.

Method - The procedure was performed with human stool specimen. Stool extraction and dilution– It was done using the EliA^{tm} Stool Extraction Kit. The extract could be stored at room temperature for max. 6 h, at 4°C for upto 30 h, and at ≤ -20°C for upto 3 months. Samples were diluted with EliA^{tm} Sample Diluent. A 1:100 dilution of the samples was required for the EliA^{tm} Calprotectin Test.

Interpretation - Phadia 100 measures calprotectin concentrations in ng/ml. By using a conversion factor given by the lot-specific code of the EliA^{tm} Calprotectin Well, the results were automatically converted to μg/g. The measuring range (detection limit, upper limit) for EliA^{tm} Calprotectin is from 15.0 to ≥ 3000 μg/g.

OUTCOME:

- Frequency of small intestinal bacterial overgrowth (SIBO) in patients with irritable bowel syndrome (IBS).
- Proportion of IBS patients that had evidence of intestinal inflammation (i.e. raised stool calprotectin).
- Co-relation between SIBO and fecal calprotectin in patients with IBS.

STATISTICAL ANALYSIS: Data were described in terms of range; mean ± standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups was done using Student t-test for independent samples for parametric data. For comparing categorical data, Chi square ($\chi 2$) test was performed and exact test was used when the expected frequency is less than 5. Pearson Correlation was also measured. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

OBSERVATION AND RESULTS

Table 1: Demographic and clinical parameters of the patients included in the study (n=112)

	Mean	SD	Minimum	Maximum
Age	35.8	11.9	19.0	60.0
Duration (years)	2.7	2.5	0.5	12.0
BMI	24.7	4.1	14.8	35.4

Total 112 IBS patients were included in the study based on Rome IV [20] criteria. Mean age of the patients was 35.8 ± 11.9 years, mean duration of symptoms was 2.7 ± 2.5 years. Mean BMI of the study patients was 24.7 ± 4.1 kg/m^2.

Table 2: Distribution of IBS population according to age

Age group (years)	No. of cases	Percentage
18-30	51	45.5%
31-40	20	17.9%
41-50	23	20.5%
51-60	18	16.1%
Total	112	100.0%

Age-wise distribution of the IBS patients in present study showed that highest number of patients i.e., 45.5% (51/112) were present in age group of 18-30 years. 17.9% (20/112) of patients were present in age group of 31-40 years, 20.5% (23/112) of patients were present in age group 41-50 years and 16.1% (18/112) of patients were present in age group 51 – 60 years.

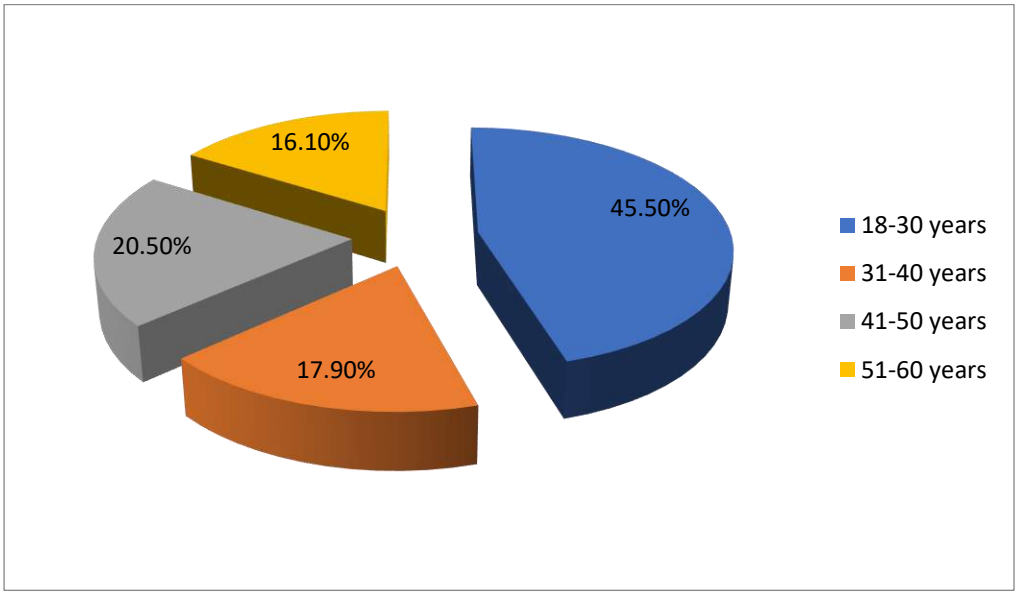

Figure 1: Distribution of IBS population according to age

Table 3: Distribution of IBS patients according to gender

Gender	No. of cases	Percentage
Female	38	33.9%
Male	74	66.1%
Total	112	100.0%

Gender-wise distribution of the IBS patients showed that 33.9% (38/112) of the patients were females and 66.1% (74/112) of the patients were males.

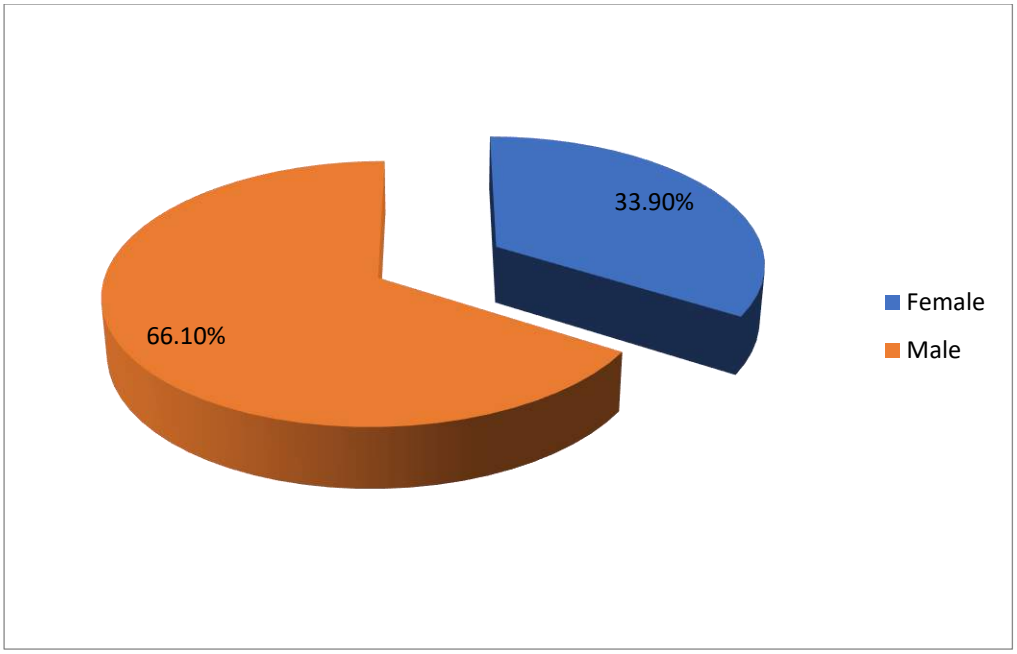

Figure 2: Distribution of IBS patients according to gender

Table 4: Distribution of IBS patients according to duration of disease

Duration (years)	No. of cases	Percentage
0-1	37	33.0%
1-2	33	29.5%
2-3	17	15.2%
> 3	25	22.3%
Total	112	100.0%

Majority of the patients i.e., 33% (37/112) if total IBS patients were those with duration less than 1 year, followed by 1-2 years (33/112), followed by >3 years (25/112), and lastly those with duration 2-3 years (17/112).

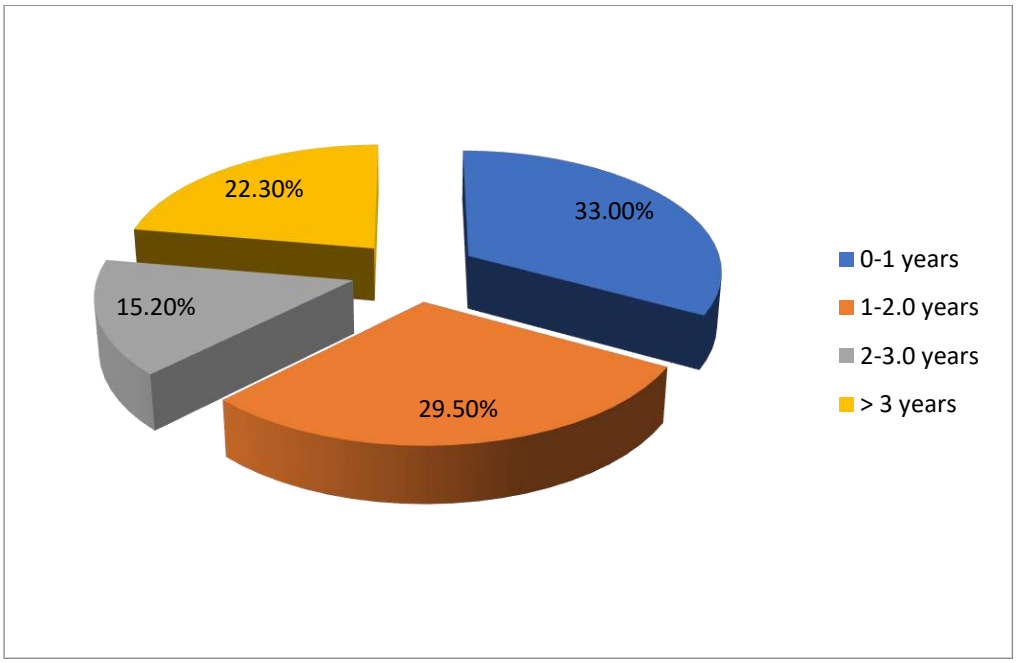

Figure 3: Distribution of IBS patients according to duration of disease

Table 5: Distribution of IBS patients according to BMI (Asian criteria)[102]

BMI group	BMI Class	No. of cases	Percentage
< 18.5	Underweight	11	9.8%
18.5-22.9	Normal Range	20	17.9%
23-24.9	Overweight	31	27.7%
25-29.9	Obese – I	38	33.9%
> 30	Obese – II	12	10.7%
Total	112	112	100.0%

While studying IBS patients based on BMI, majority of the patients, i.e., 33.9% (38/112) belonged to 'Obese – I' class. If we combine obese - I and obese – II classes, IBS patients can be categorized into obese vs non-obese, i.e., 44.6% (50/112) vs 55.4% (62/112), respectively.

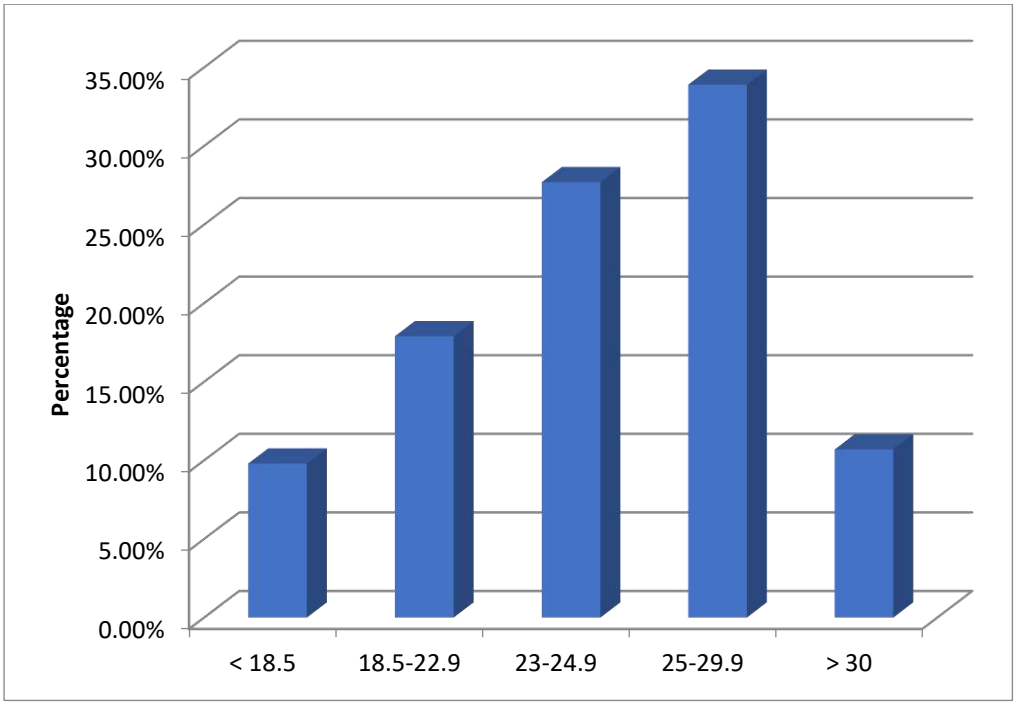

Figure 4: Distribution of IBS patients according to BMI (Asian criteria)

Table 6: Gender-wise distribution of demographic and clinical parameters of the patients included in the study

Parameter	Male		Female		t	p-value
	Mean	SD	Mean	SD		
Age	34.4	11.9	38.5	11.6	-1.738	0.085
Duration (years)	2.7	2.5	2.8	2.5	-0.284	0.777
BMI	25.2	3.7	23.6	4.6	2.027	0.045

Males and females had comparable age and duration of disease ($p = 0.085$ and $p = 0.777$, respectively). BMI of males was significantly higher than females ($p = 0.045$).

Table 7: Distribution according to subtypes of IBS

IBS Sub-type	No. of cases	Percentage
IBS-C	21	18.8%
IBS-D	80	71.4%
IBS-M	11	9.8%
Total	112	100.0%

Among the three sub-types of IBS, i.e., constipation predominant IBS (IBS-C), diarrhea predominant IBS (IBS-D) and mixed type IBS (IBS-M); most common sub-type of IBS in the present study was IBS-D (71.4%), followed by IBS-C (18.8%) and lastly IBS-M (9.8%).

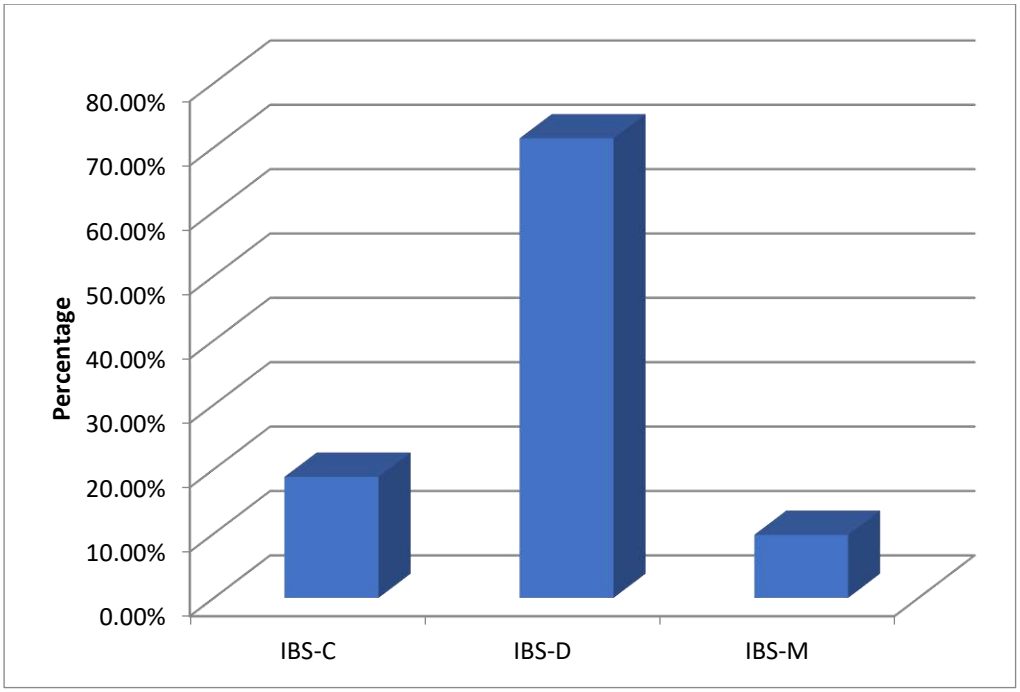

Figure 5: Distribution according to subtypes of IBS

Table 8: Clinical and psychological assessment scores in IBS patients (n=112)

Clinical and Psychological scores	Mean	SD	Minimum	Maximum
IBS-SSS	215.2	65.6	110.0	360.0
IBS-QOL	66.7	15.0	17.6	95.5
HAM-A	15.3	5.9	3.0	34.0
HAM-D	10.9	5.0	3.0	21.0

In IBS patients, mean IBS-SSS score, that indicates severity of symptoms was found to be 215.2 ± 66.6 and mean IBS-QOL score, which estimates IBS specific quality of life was found to be 66.7 ± 15.0. Mean HAM-A score to evaluate anxiety, was found to be 15.3 ± 5.9 and mean HAM-D score to assess depression was found to be 10.9 ± 5.0.

Table 9: Classification of the IBS patients based on severity of symptoms (IBS-SSS)[14]

IBS-SSS	Severity	'n'	Percentage
75-175	Mild	38	33.9%
175-300	Moderate	59	52.7%
> 300	Severe	15	13.4%
Total		112	100%

Among 112 IBS patients, 33.9% (38/112) patients had mild disease, 52.7% (59/112) patients had moderate disease and 13.4% (15/112) patients had severe disease.

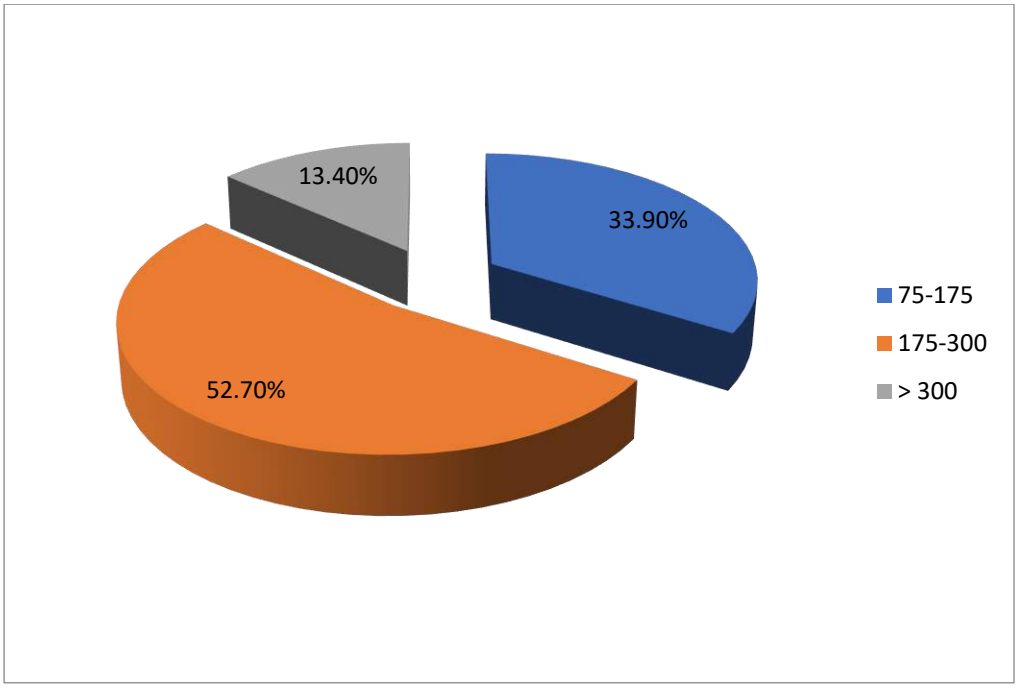

Figure 6: Classification of the IBS patients based on severity of symptoms (IBS-SSS)

Table 10: Gender-wise distribution of clinical and psychological parameters in IBS

Gender	Male		Female		t	p-value
	Mean	SD	Mean	SD		
IBS-SSS	214.1	67.6	217.3	62.3	-0.242	0.809
IBS-QOL	67.8	14.4	64.5	16.2	1.110	0.270

Mean IBS-SSS score among males was 214.3 ± 67.6 and among females was 217.3 ± 62.3, (p=0.809). Mean IBS-QOL score among males was 67.8 ± 14.4 and among females was 64.5 ± 16.2, (p=0.270).

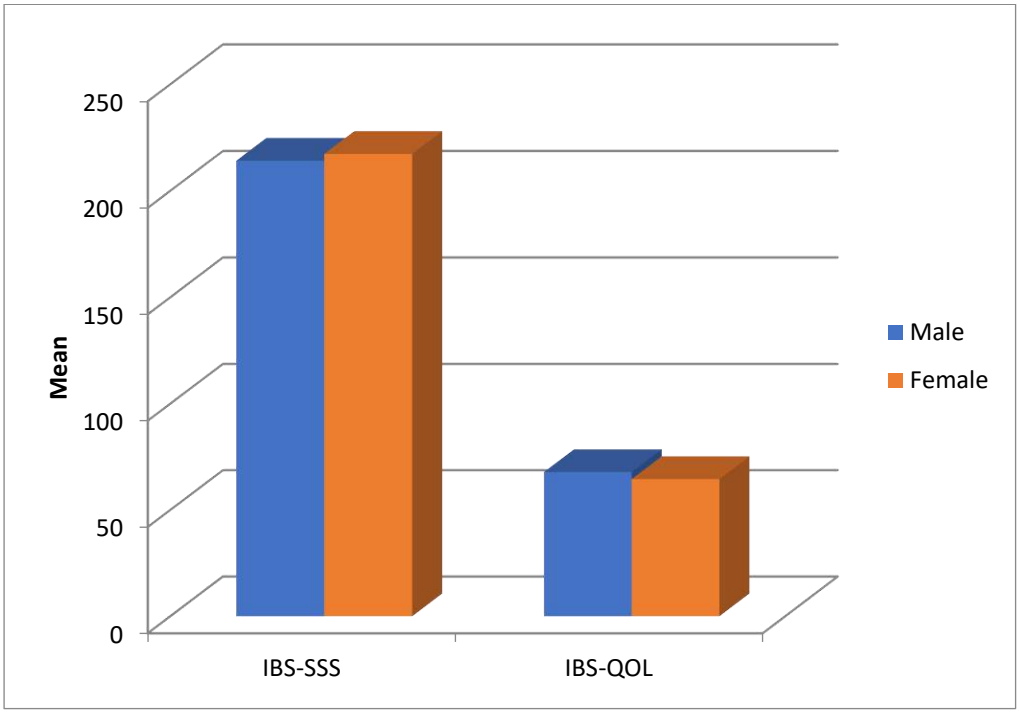

Figure 7: Gender-wise distribution of clinical and psychological parameters in IBS

Table 11: Correlation between duration of IBS and clinical & psychological parameters

		IBS-SSS	IBS-QOL	HAM-A	HAM-D
Duration (years)	Pearson Correlation	0.296	-0.294	0.160	0.093
	p-value	0.002	0.002	0.091	0.332
	N	112	112	112	112

Duration of disease had significant impact on the IBS-SSS and IBS-QOL score ($p < 0.002$). Duration of disease had no significant effect on anxiety or depression in IBS patients, ($p = 0.091$), and ($p = 0.332$), respectively.

Table 12: Effect of BMI on clinical and psychological parameters in IBS

		IBS-SSS	IBS-QOL	HAM-A	HAM-D
BMI	Pearson Correlation	0.058	-0.059	0.019	-0.002
	p-value	0.543	0.539	0.841	0.981
	N	112	112	112	112

BMI had no significant impact on clinical and psychological parameters in IBS patients.

Table 13: Prevalence of anxiety in IBS patients

HAM-A Score	No. of cases	Percentage
HAM-A ≤ 7 (Anxiety Absent)	17	15.2%
HAM-A >7 (Anxiety Present)	95	84.8%
Total	112	100.0%

Anxiety was present in 84.8% of the IBS patients.

Table 14: Prevalence of depression in IBS patients

HAM-D Score	No. of cases	Percentage
HAM-D ≤ 7 (Depression Absent)	37	33.0%
HAM-D > 7 (Depression Present)	75	67.0%
Total	112	100.0%

Depression was present in 67.0 % of the IBS patients.

Table 15: Prevalence of SIBO in IBS

SIBO	No. of cases	Percentage
Negative	76	67.9%
positive	36	32.1%
Total	112	100.0%

SIBO was present in 32.1% (36/112) of the IBS patients.

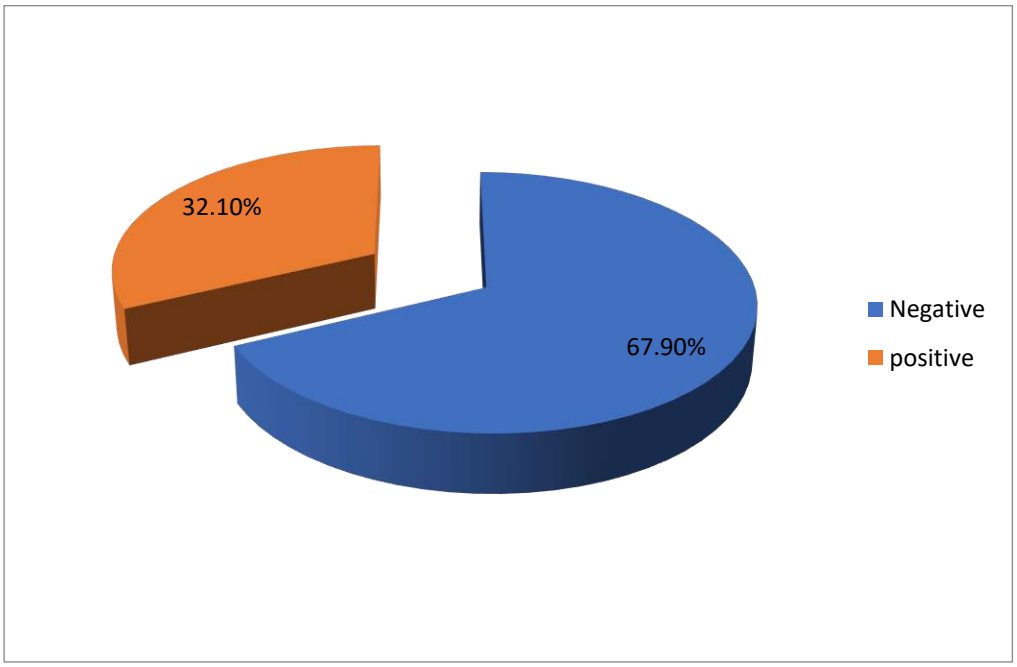

Figure 8: Prevalence of SIBO in IBS

Table 16: Prevalence of SIBO according to sub-type of IBS

IBS sub-types	SIBO				Total	Chi-square value	p-value
	Negative		Positive				
IBS-C	19	25.0%	2	5.6%	21	6.647	0.036
IBS-D	49	64.5%	31	86.1%	80		
IBS-M	8	10.5%	3	8.3%	11		
Total	76	100.0%	36	100.0%	112		

While comparing the prevalence of SIBO among three sub-types of IBS, IBS-D had the highest prevalence. And the difference was statistically significant (p = 0.036).

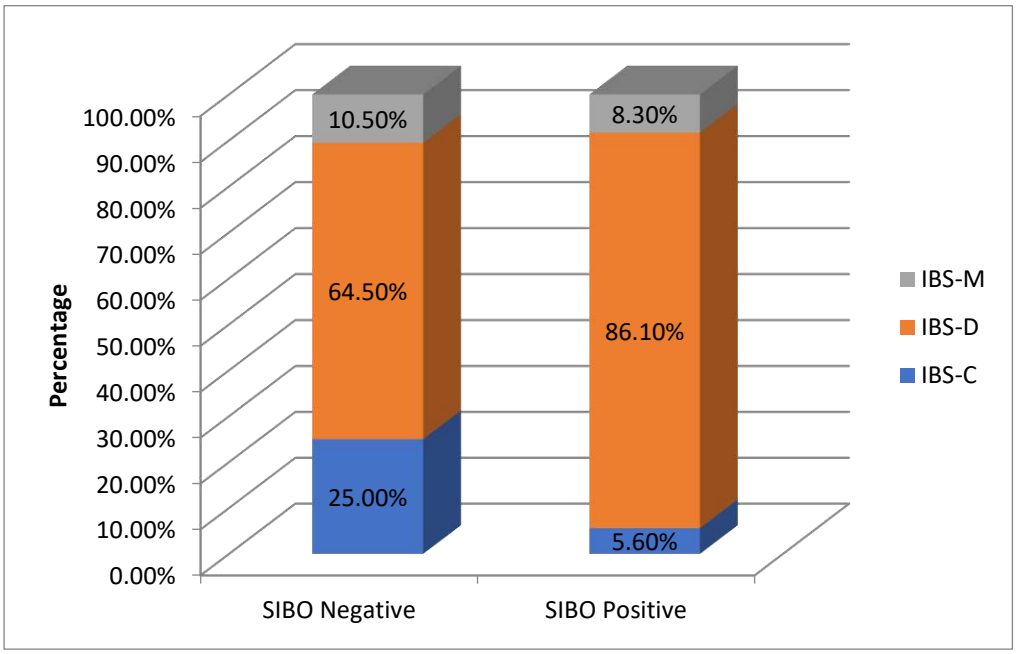

Figure 9: Prevalence of SIBO according to sub-type of IBS

Table 17: Effect of Age on SIBO

	SIBO				t	p-value
	Negative		Positive			
Age (years)	Mean	SD	Mean	SD	-1.373	0.173
	34.7	11.7	38.0	12.2		

There was no significant effect of age on SIBO, (p = 0.173).

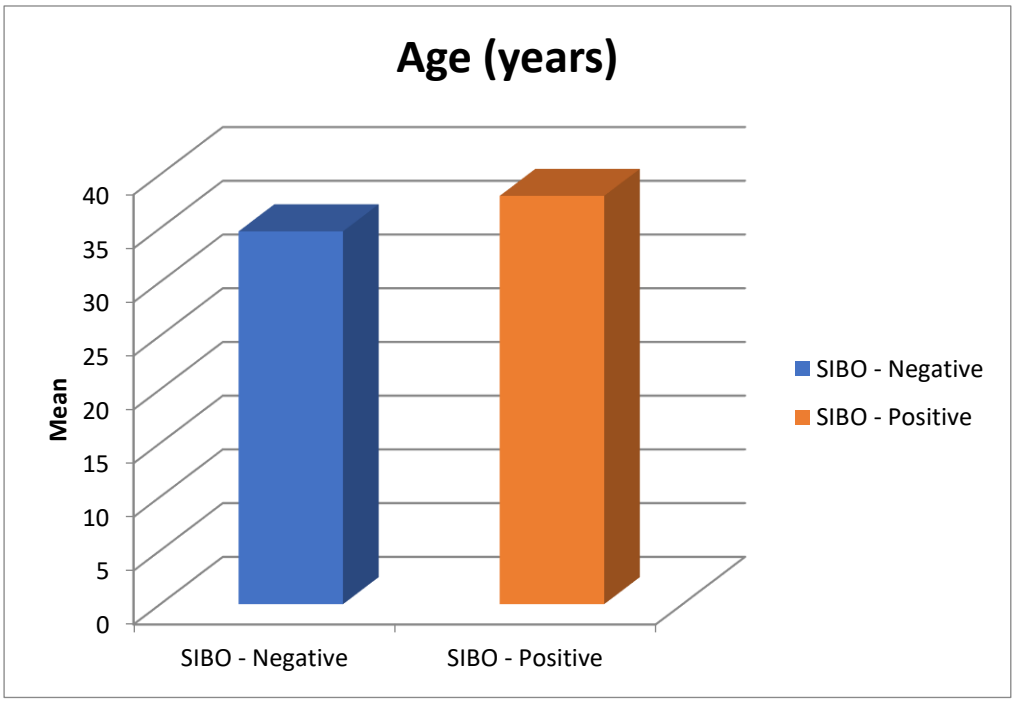

Figure 10: Effect of Age on SIBO

Table 18: Gender-wise distribution of SIBO

	SIBO				Total	Chi-square value	p-value
Gender	Negative		Positive				
F	23	30.3%	15	41.7%	38	1.417	0.234
M	53	69.7%	21	58.3%	74		
Total	76	100.0%	36	100.0%	112		

SIBO was found to be higher in males as compared to females, but the difference was not significant, (p = 0.234).

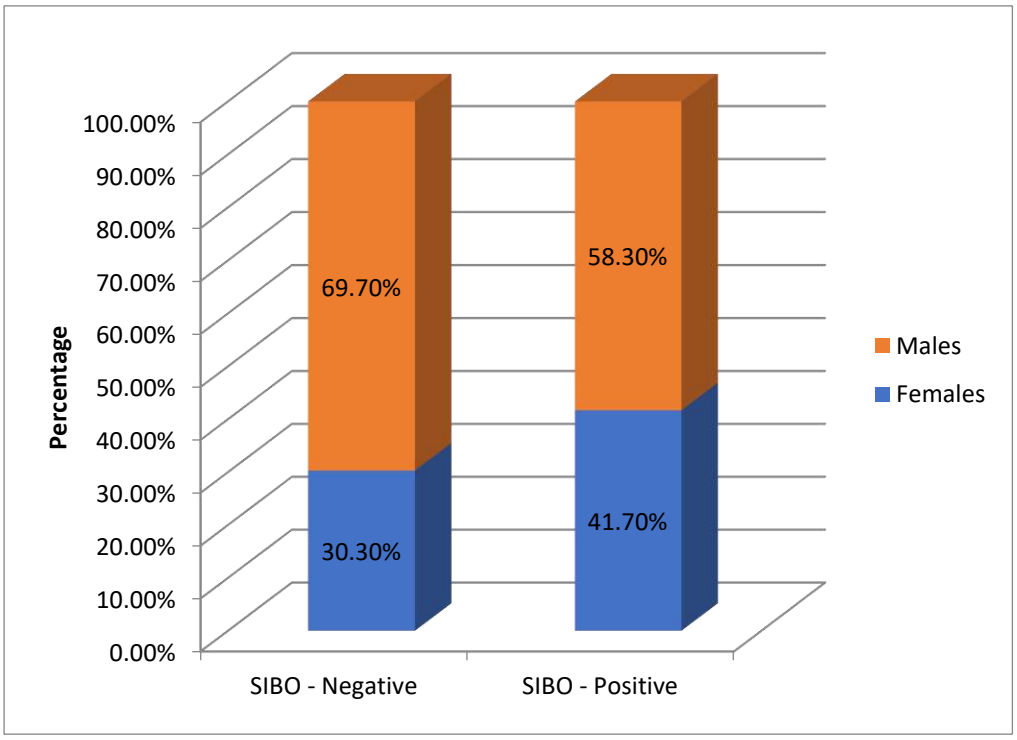

Figure 11: Gender-wise distribution of SIBO

Table 19: Effect of duration of disease on SIBO

SIBO	Negative		Positive		t	p-value
	Mean	SD	Mean	SD		
Duration (years)	2.2	2.3	3.9	2.7	-3.450	0.001

Patients who had longer duration of disease, were significantly more likely to have SIBO. (p = 0.001)

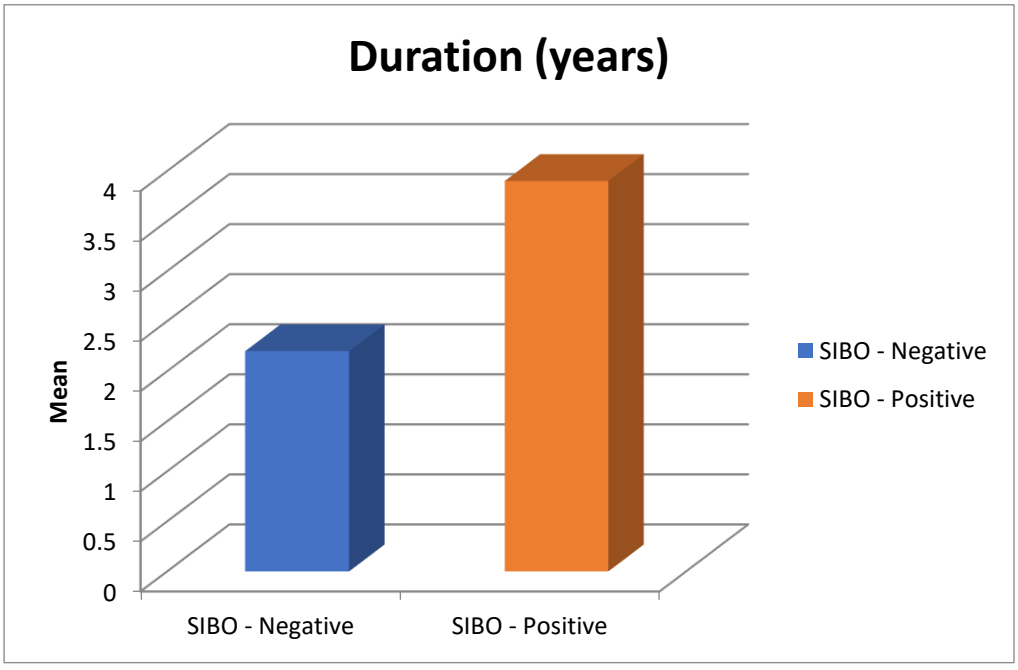

Figure 12: Effect of duration of disease on SIBO

Table 20: Effect of BMI on SIBO

SIBO	Negative		Positive		t	p-value
	Mean	SD	Mean	SD		
BMI (Kg/m^2)	24.4	3.9	25.3	4.5	-1.036	0.303

Higher BMI was associated with higher prevalence of SIBO, but the correlation was not significant. (p = 0.303)

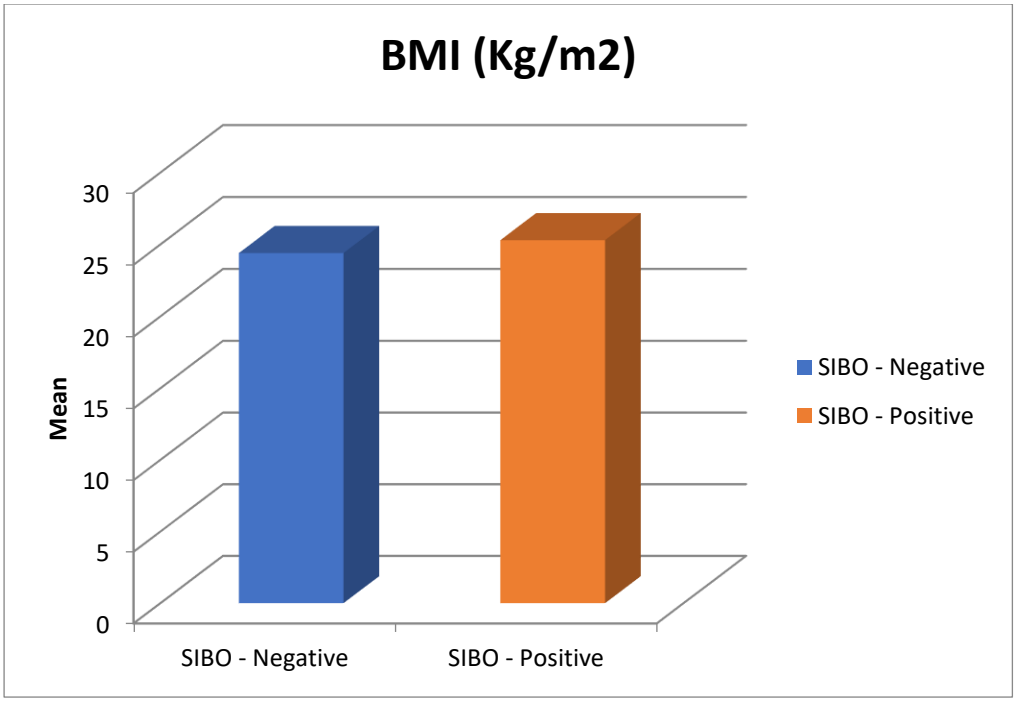

Figure 13: Effect of BMI on SIBO

Table 21: Effect of SIBO on severity of symptoms and quality of life

SIBO	Negative		Positive		t	p-value
	Mean	SD	Mean	SD		
IBS-SSS	185.1	46.8	278.8	52.9	-9.476	0.000
IBS-QOL	72.7	10.5	53.9	15.3	7.550	0.000

IBS-SSS, i.e., Symptom severity score was significantly higher in patients who tested positive for SIBO ($p<0.001$). IBS-QOL, which measures quality of life in IBS patients (higher is better), was significantly lower in SIBO positive patients ($p<0.001$).

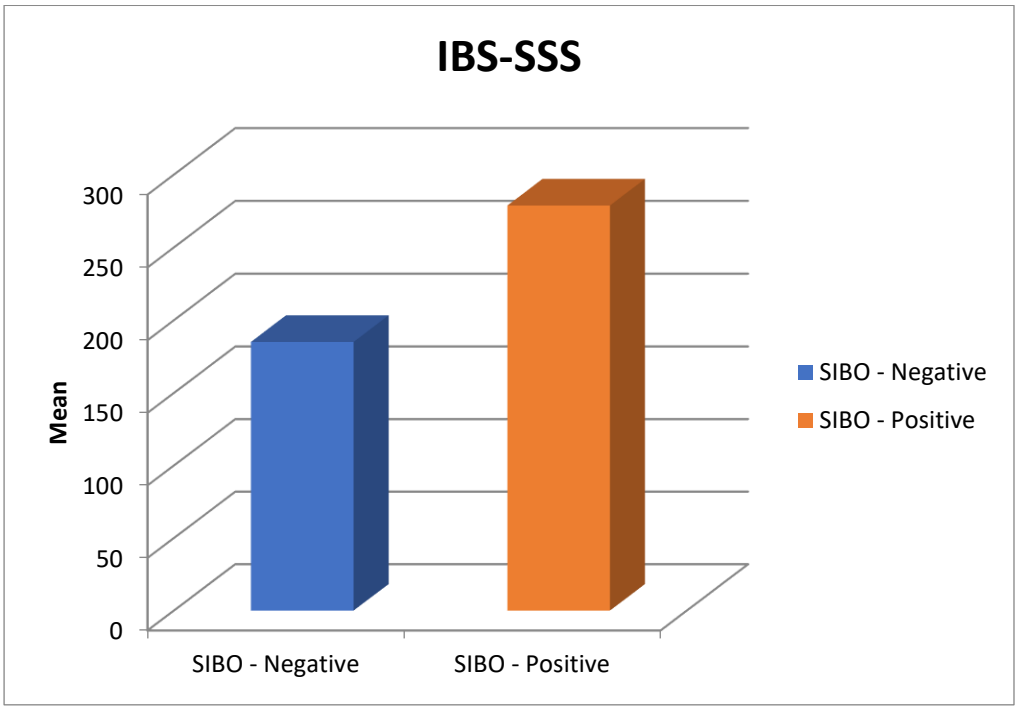

Figure 14: Effect of SIBO on severity of symptoms (IBS-SSS)

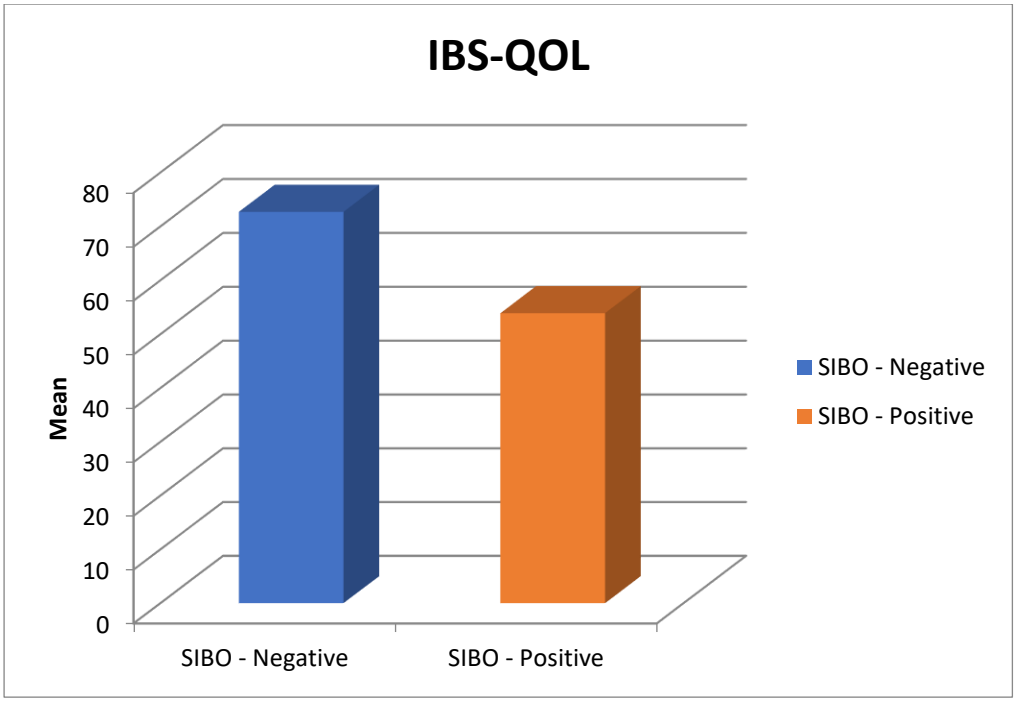

Figure 15: Effect of SIBO on quality of life (IBS-QOL)

Table 22: Effect of SIBO on laboratory parameters

SIBO	Negative		Positive		t	p-value
	Mean	SD	Mean	SD		
Haemoglobin (g/dL)	13.5	1.3	13.2	1.6	0.924	0.357
Creatinine (mg/dL)	0.7	0.2	0.8	0.2	-1.057	0.293
ALT (IU/L)	30.0	11.0	34.8	21.9	-1.571	0.119
CRP (mg/L)	2.0	1.1	3.9	2.0	-6.467	0.000

Among the laboratory parameters studied in IBS patients, CRP levels correlated significantly with presence of SIBO ($p < 0.001$).

Table 23: Distribution of patients according to fecal calprotectin levels

Fecal calprotectin (μg/g)	No. of cases	Percentage
0-50	81	72.3%
50-100	26	23.2%
100-150	2	1.8%
150-200	3	2.7%
Total	112	100%

72.3% (81/112) of IBS patients had fecal calprotectin level between 0-50 μg/g; 23.2% (26/112) patients had fecal calprotectin between 50-100 μg/g; 1.8% (2/112) patients and 2.7% (3/112) patients had fecal calprotectin between 100-150 & 150-200, respectively. Maximum value of fecal calprotectin in our study was 167 μg/g.

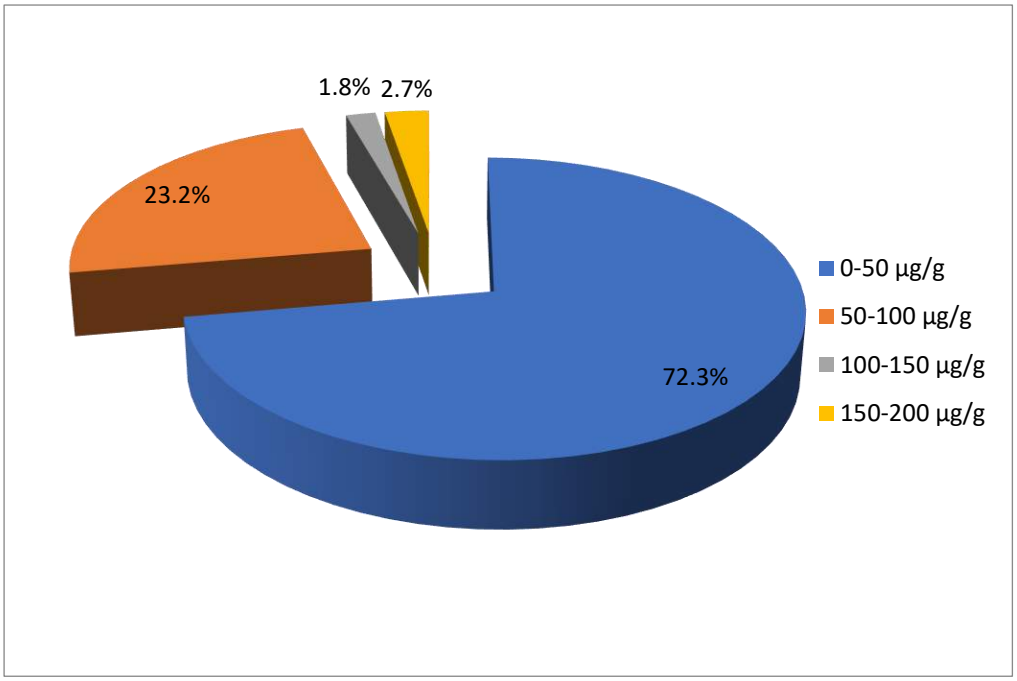

Figure 16: Distribution of patients according to fecal calprotectin levels

Table 24: Prevalence of intestinal inflammation in IBS

Fecal calprotectin (μg/g)	Value
Mean ± SD	41.6 ± 27.2
<50	81 (72.3%)
> 50	31 (27.7%)
Total	112 (100.0%)

Mean fecal calprotectin level in the study population came out to be 41.6 ± 27.2 μg/g, and its cut-off value to define raised (intestinal inflammation) vs normal fecal calprotectin level was taken as 50 μg/g. 27.7 % (31/112) of the IBS patients in this study were found to have raised fecal calprotectin level.

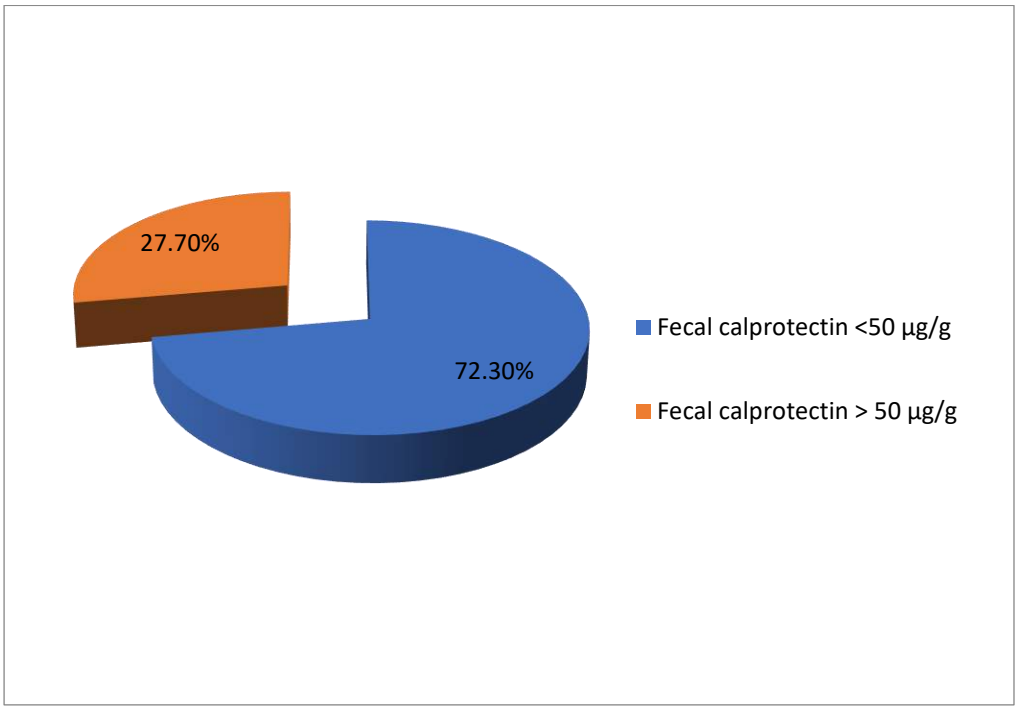

Figure 17: Prevalence of intestinal inflammation in IBS

Table 25: Prevalence of intestinal inflammation according to IBS-subtypes

	Fecal calprotectin (µg/g)				Total	Chi-square value	p-value
IBS type	< 50		> 50				
IBS-C	20	24.7%	1	3.2%	21	6.984	0.030
IBS-D	53	65.4%	27	87.1%	80		
IBS-M	8	9.9%	3	9.7%	11		
Total	81	100.0%	31	100.0%	112		

On comparing the presence of intestinal inflammation among three sub-types of IBS, IBS-D had the highest prevalence of 87.1% (80/112). And the difference was statistically significant (p 0.030).

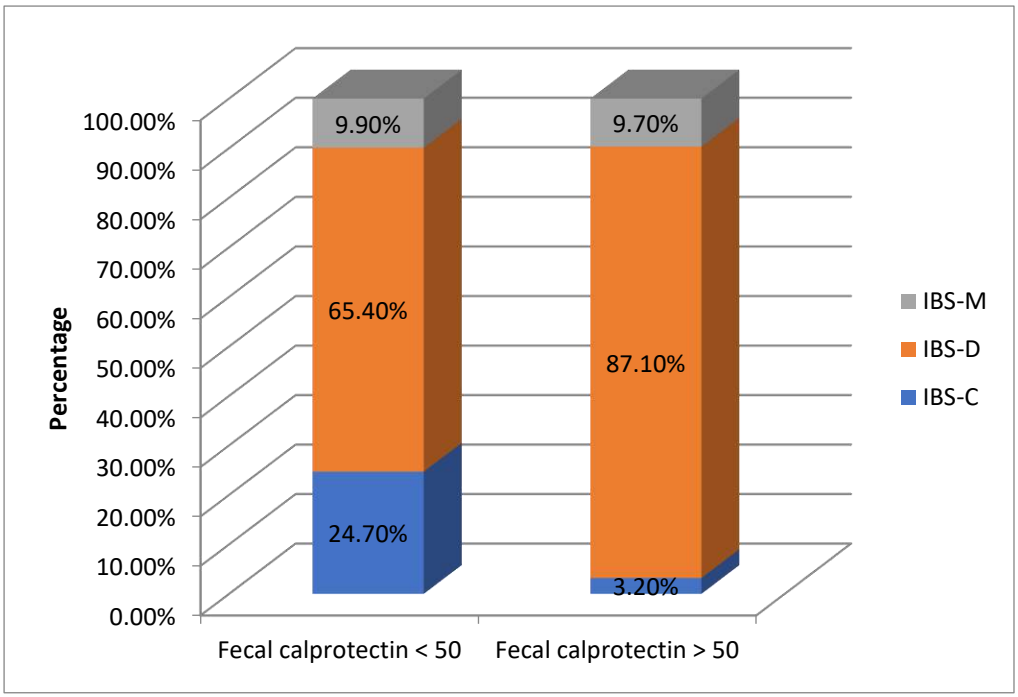

Figure 18: Prevalence of intestinal inflammation according to IBS-subtypes

Table 26: Effect of Age on intestinal inflammation

Fecal calprotectin (µg/g)	< 50		> 50		t	p-value
	Mean	SD	Mean	SD		
Age (years)	35.4	12.4	36.8	10.7	-0.578	0.564

There was no significant effect of age on intestinal inflammation (p = 0.564).

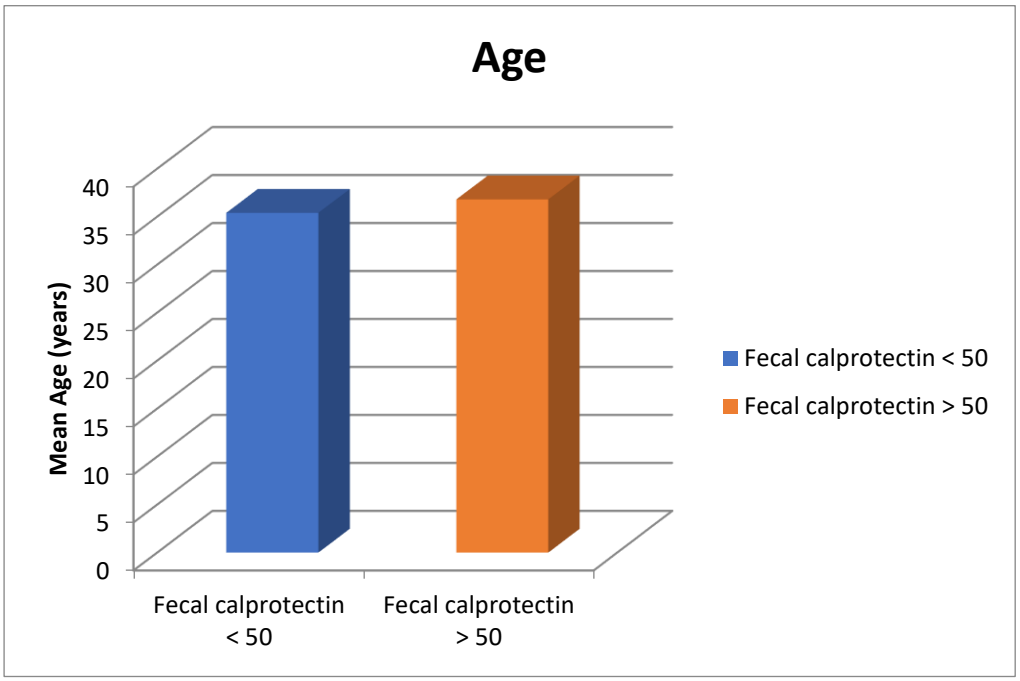

Figure 19: Effect of Age on intestinal inflammation

Table 27: Gender-wise distribution of intestinal inflammation

	Fecal calprotectin (μg/g)				Total	Chi-square value	p-value
Sex	< 50		> 50				
Females	25	30.9%	13	41.9%	38	1.226	0.268
Males	56	69.1%	18	58.1%	74		
Total	81	100.0%	31	100.0%	112		

Intestinal inflammation was more prevalent in males, but the difference was not statistically significant (p = 0.268).

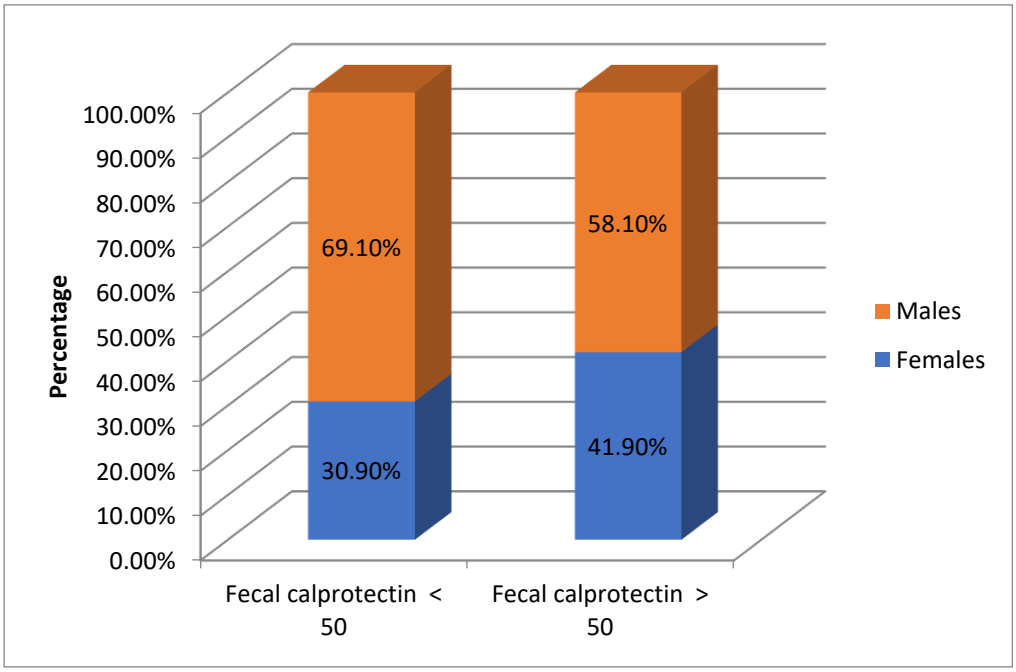

Figure 20: Gender-wise distribution of intestinal inflammation

Table 28: Effect of duration of disease on intestinal inflammation

Fecal calprotectin	< 50		> 50		t	p-value
	Mean	SD	Mean	SD		
Duration (years)	2.4	2.5	3.7	2.3	-2.506	0.014

Patients with longer duration of disease, tend to have significantly higher prevalence of intestinal inflammation. (p = 0.014)

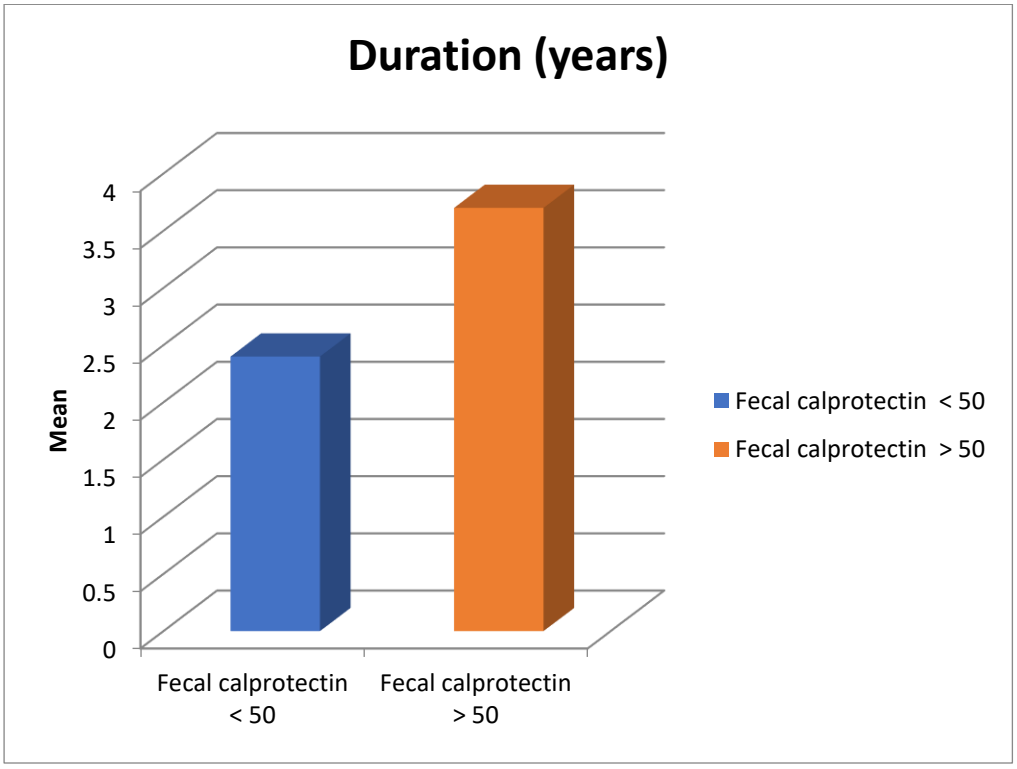

Figure 21: Effect of duration of disease on intestinal inflammation

Table 29: Effect of BMI on intestinal inflammation

Fecal calprotectin	< 50		> 50		t	p-value
	Mean	SD	Mean	SD		
BMI (kg/m^2)	24.5	3.8	25.1	4.9	-0.618	0.538

Although mean BMI was higher in patients with raised fecal calprotectin as compared to patients with normal fecal calprotectin, but the difference was not statistically significant (p = 0.538).

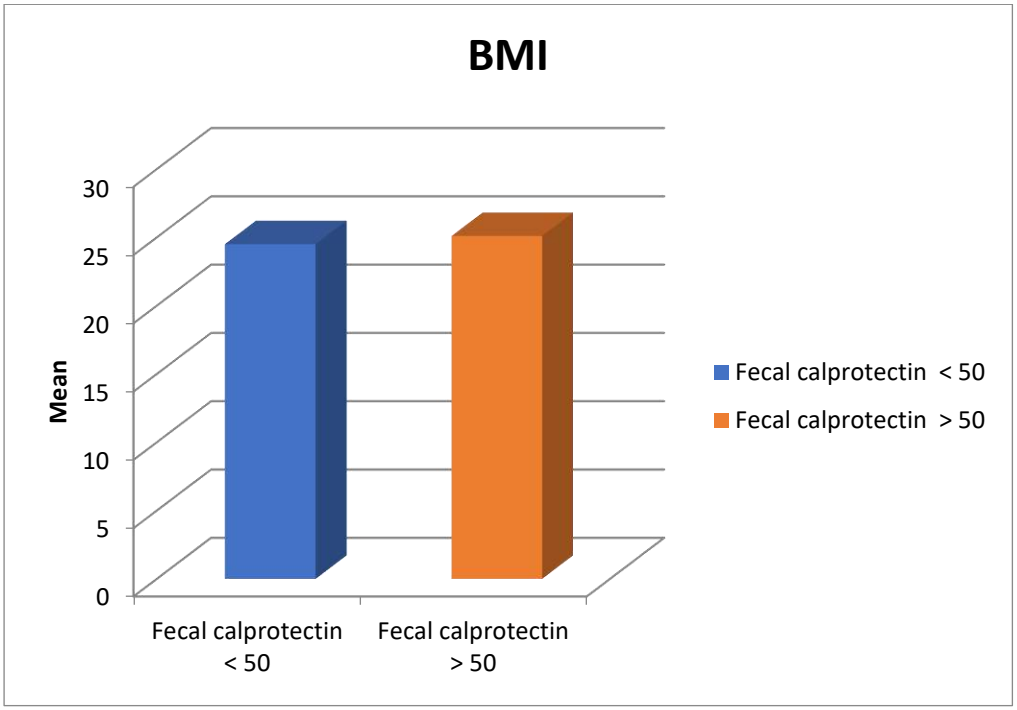

Figure 22: Effect of BMI on intestinal inflammation

Table 30: Effect of intestinal inflammation on severity of symptoms and quality of life

Fecal calprotectin (µg/g)	< 50		> 50		t	p-value
	Mean	SD	Mean	SD		
IBS-SSS	187.4	48.3	288.0	46.0	-9.985	0.000
IBS-QOL	72.1	11.2	52.4	14.6	7.618	0.000

IBS-SSS, i.e., Symptom severity score was significantly higher in patients who had raised fecal calprotectin level ($p < 0.001$). IBS-QOL, which measures quality of life in IBS patients (higher is better), was significantly lower in patients who had raised fecal calprotectin level ($p < 0.001$)

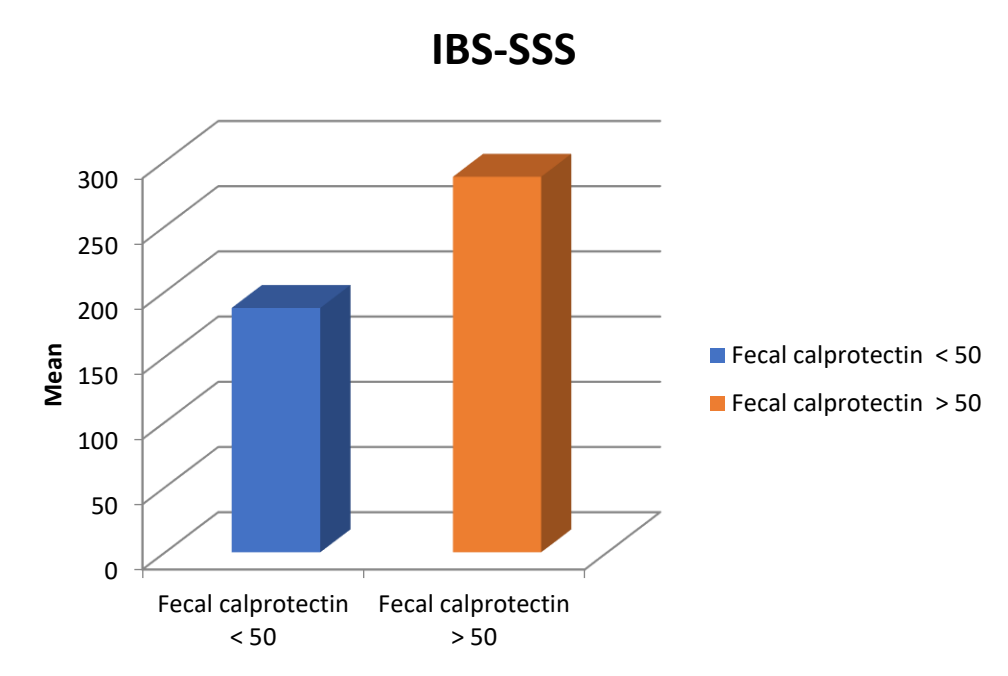

Figure 23: Effect of intestinal inflammation on severity of symptoms (IBS-SSS)

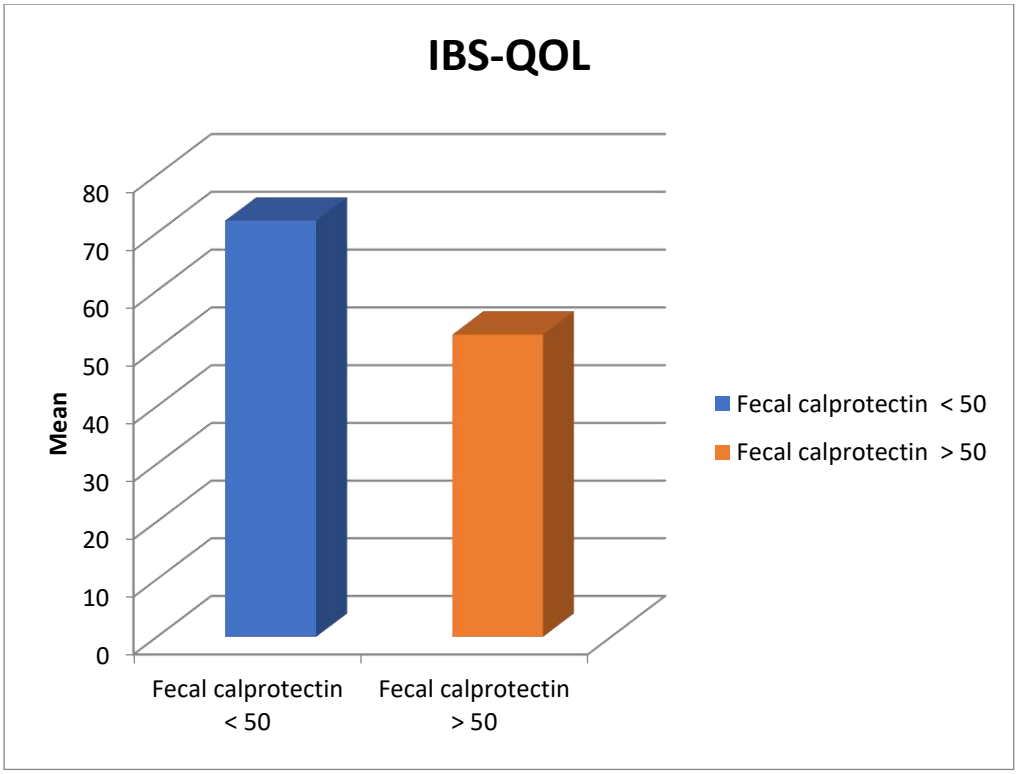

Figure 24: Effect of intestinal inflammation on quality of life (IBS-QOL)

Table 31: Effect of intestinal inflammation on laboratory parameters

Fecal calprotectin (μg/g)	< 50		> 50		t	p-value
	Mean	SD	Mean	SD		
Haemoglobin (g/dL)	13.530	1.442	13.177	1.486	1.147	0.254
Creatinine (mg/dL)	0.759	0.199	0.839	0.219	-1.845	0.068
ALT (IU/L)	30.086	11.89+1	35.452	22.095	-1.654	0.101
CRP (mg/L)	2.073	1.274	4.085	1.912	-6.453	0.000

In patients with raised fecal calprotectin i.e., with intestinal inflammation, CRP level was significantly elevated, $p < 0.001$ and $p = 0.035$, respectively.

Table 32: Correlation between SIBO and intestinal inflammation in IBS patients

	SIBO				Total	Chi-square value	p-value
Fecal calprotectin (µg/g)	Negative		Positive				
< 50	74	97.4%	7	19.4%	81	74.102	0.0001
> 50	2	2.6%	29	80.6%	31		
Total	76	100.0%	36	100.0%	112		

Out of 36 patients who had SIBO, fecal calprotectin was raised in 29 patients, which translates to 80.6%. Significantly higher number of SIBO positive IBS patients had raised fecal calprotectin levels as compared to those with SIBO negative. (p = 0.001)

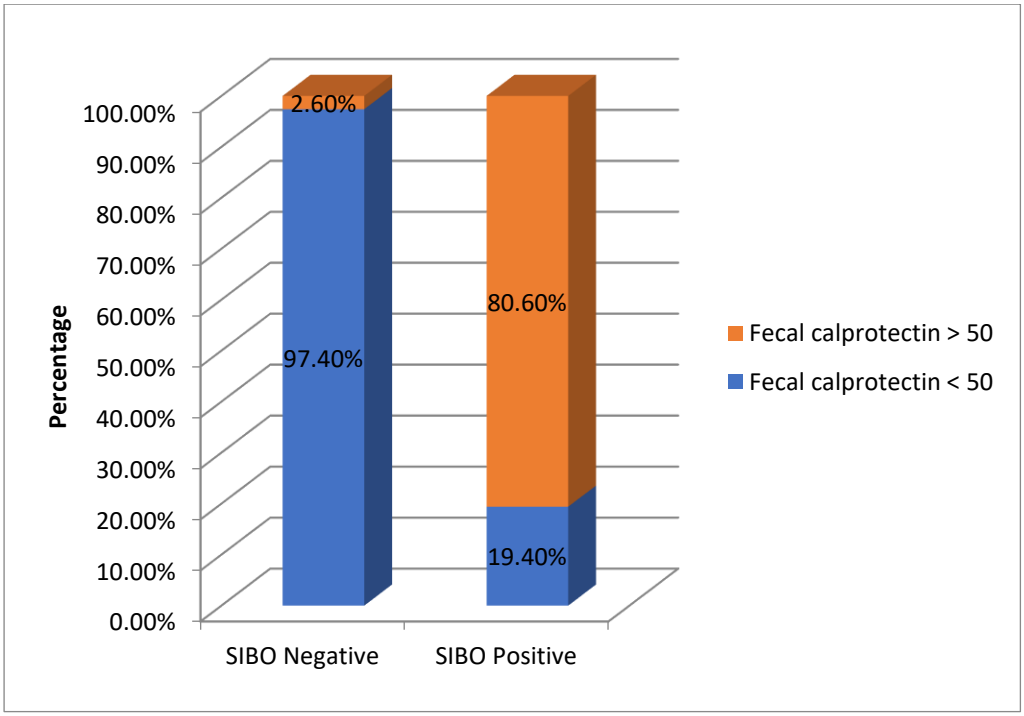

Table 25: Correlation between SIBO and intestinal inflammation in IBS patients

Follow-up:

IBS patients who were positive for SIBO or were having raised fecal calprotectin or both, underwent colonoscopy to rule out organic disease like IBD. None of the patients who underwent colonoscopy were found to have organic disease. Such patients received rifaximin by the primary treating team in dose of 550mg three times a day for 2 weeks. After 12 weeks of initial enrolment, all IBS patients were re-evaluated for assessment of change in IBS-SSS and IBS-QOL scores. A decrease of 50 points in IBS-SSS was associated with a clinically meaningful improvement.[52] An improvement of 14 points in IBS-QOL translates to a clinically meaningful improvement.[56]

Table 33: Follow-up assessment of symptom severity in IBS patients with SIBO

Change in IBS-SSS	SIBO				Total	Chi-square value	p-value
	SIBO Negative		SIBO Positive				
< 50	23	30.3%	5	13.9%	28	3.493	0.067
> 50	53	69.7%	31	86.1%	84		
Total	76	100.0%	36	100.0%	112		

In patients who had SIBO at baseline (n=36), IBS-SSS improved in 86.1% (31/36). (p=0.067)

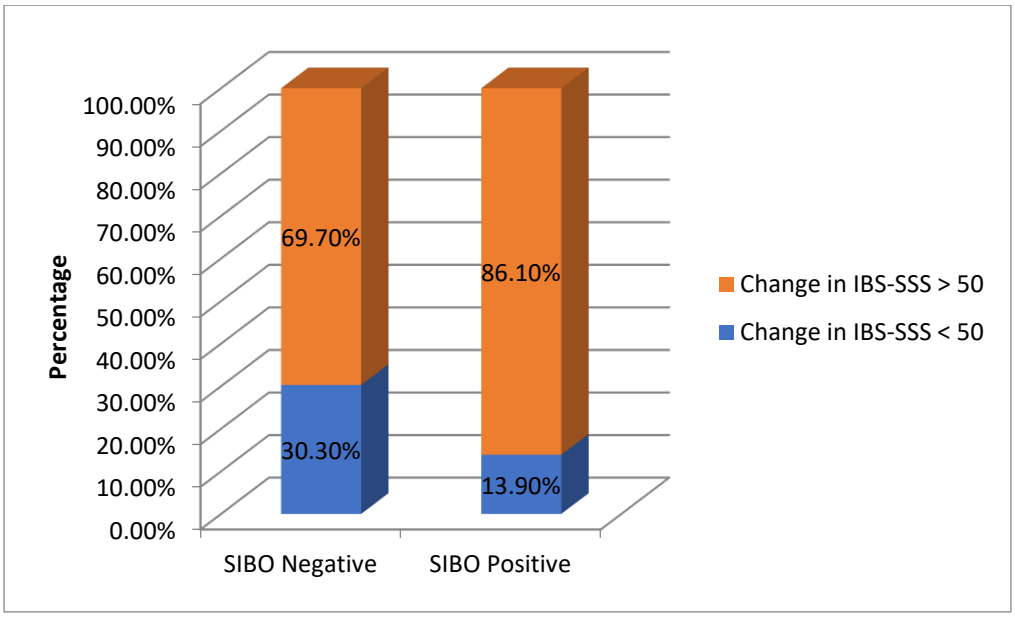

Figure 26: Follow-up assessment of symptom severity in IBS patients with SIBO

Table 34: Follow-up assessment of quality of life in IBS patients with SIBO

	SIBO				Total	Chi-square value	p-value
Change in IBS-QOL	SIBO Negative		SIBO Positive				
< 14	35	46.05%	6	16.67%	41	9.09	0.003
> 14	41	53.95%	30	83.33%	71		
Total	76	100.00%	36	100.00%	112		

In patients who had SIBO at baseline (n=36), IBS-QOL improved in 83.3% (30/36). (p=0.003)

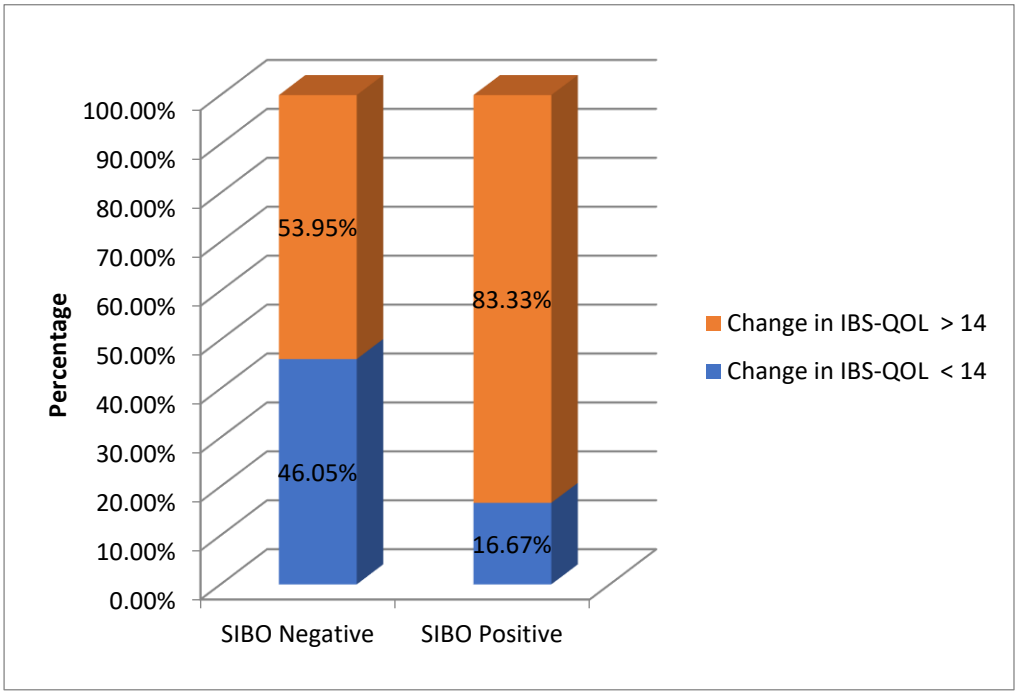

Figure 27: Follow-up assessment of quality of life in IBS patients with SIBO

Table 35: Follow-up assessment of symptom severity in IBS patients with raised fecal calprotectin

	Fecal calprotectin (μg/g)				Total	Chi-square value	p-value
Change in IBS-SSS	Fecal calprotectin < 50 μg/g		Fecal calprotectin > 50 μg/g				
< 50	25	30.9%	3	9.7%	28	5.367	0.027
> 50	56	69.1%	28	90.3%	84		
Total	81	100.0%	31	100.0%	112		

In patients who had raised fecal calprotectin at baseline (n=31), IBS-SSS improved in 90.3% (28/31). (p = 0.027)

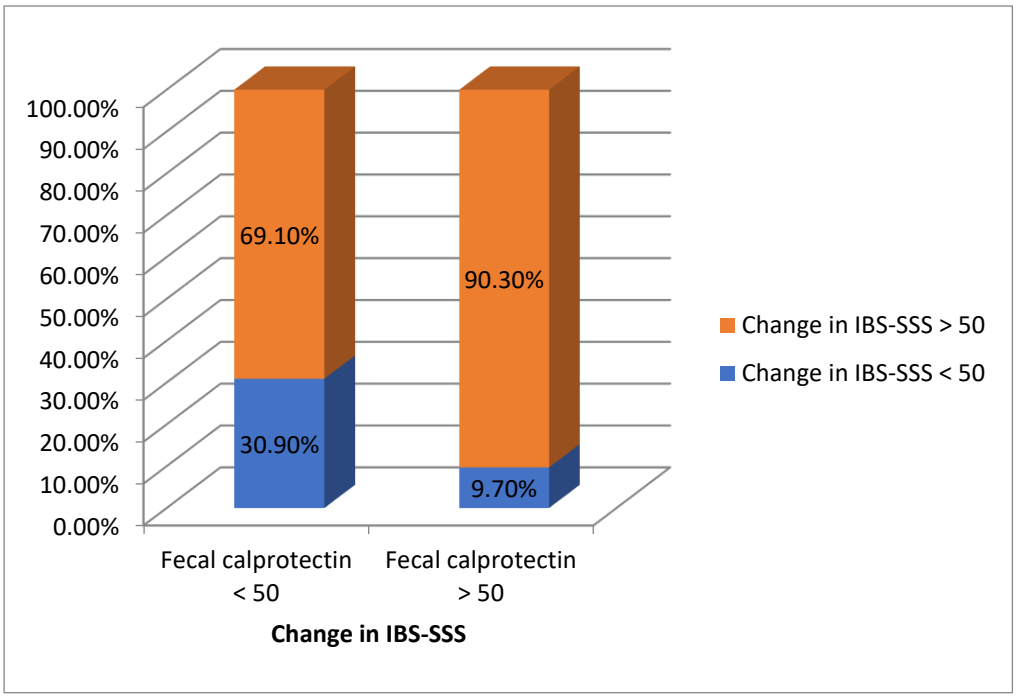

Figure 28: Follow-up assessment of symptom severity in IBS patients with raised fecal calprotectin

Table 36: Follow-up assessment of quality of life in IBS patients with raised fecal calprotectin

	Fecal calprotectin (mg/g)				Total	Chi-square value	p-value
Change in IBS-QOL	Fecal calprotectin < 50 μg/g		Fecal calprotectin > 50 μg/g				
< 14	37	45.68%	4	12.90%	41	10.378	0.001
> 14	44	54.32%	27	87.10%	71		
Total	81	100.00%	31	100.00%	112		

In patients who had raised fecal calprotectin at baseline (n=31), IBS-QOL improved in 87.1% (27/31). (p = 0.001)

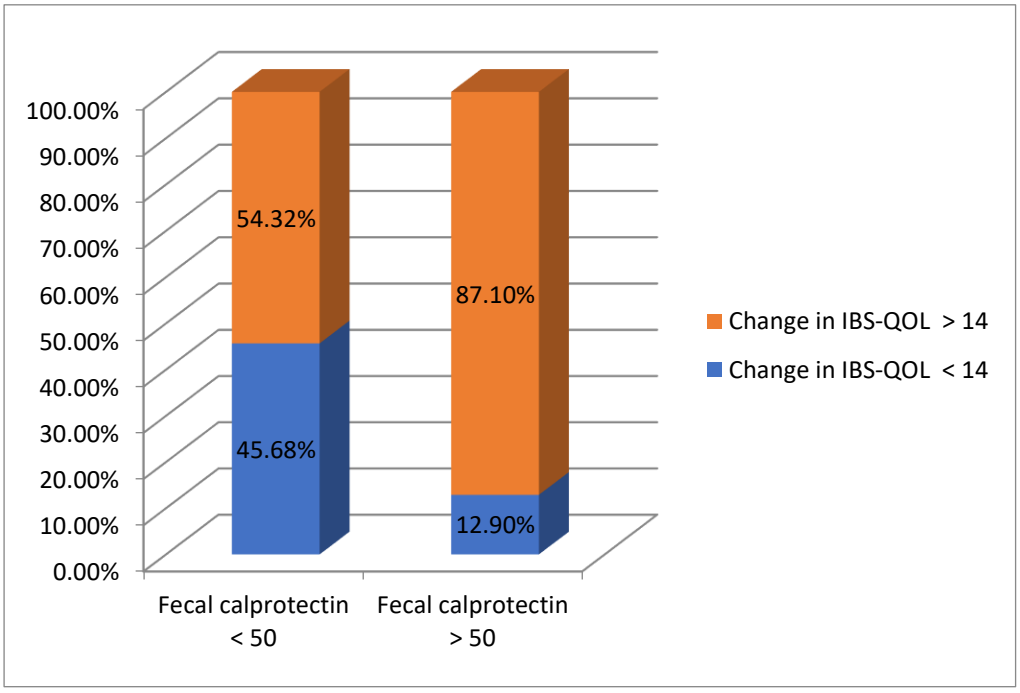

Figure 29: Follow-up assessment of quality of life in IBS patients with raised fecal calprotectin

DISCUSSION

Irritable bowel syndrome is a functional gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits in the absence of any organic cause. It is diagnosed on the basis of clinical criteria. Small intestinal bacterial overgrowth is a condition in which colonic bacteria are seen in excess in the small intestine. It is associated with bloating along with other symptoms like flatulence, abdominal discomfort, or chronic watery diarrhea. Patients with SIBO and IBS have overlapping symptoms. And, it appears that a proportion of IBS subjects have SIBO. Low levels of inflammation have also been reported in IBS. There is an evidence of increased numbers of inflammatory cells (e.g. mast cells and T cells) in both adult and paediatric IBS. Calprotectin is a major protein found in the cytosol of inflammatory cells, and its level in stool sample can be used to estimate intestinal mucosal inflammation. The use of the calprotectin level as a biomarker of intestinal inflammation could represent an interesting targeted therapeutic approach not only to rule out IBD, but also to identify potential IBS candidates for an anti-inflammatory treatment.

The present study was done to evaluate presence of SIBO and subclinical intestinal inflammation simultaneously in IBS patients. We evaluated 112 consecutive IBS patients. Their mean age was 35.8 ± 11.9 years. Majority of the patients i.e., 45.5% (51/112) were of the age between 18 to 30 years. Our study consisted of 66.1% (74/112) males as compared to 33.9% (38/112) females. Although IBS is more common in women than in men in most parts of the world.[103,104] In India, it is reported more often in males.[105] These findings have also been seen in other hospital-based studies[106,107], and show a possibility of male patients consulting doctors more often than females because of socio-cultural reasons.

Most common subtype of IBS in our population was diarrhea predominant IBS (IBS-D), with 71.4% of the patients falling in this group; other two subtypes were IBS-C having 18.8% patients and IBS-M having

9.8%. Sachdeva et al[92] studied 59 consecutive patients with IBS in a tertiary care hospital, of which 27 (45.7%) were diarrhea predominant (D-IBS), 11 (18.6%) were constipation predominant (C-IBS) & 21 (35.5%) were mixed type (M-IBS). Goyal et al[26] also found diarrhea predominant subtype (IBS-D) as the most common subtype (63.0%), while 27.2% were IBS-C and 8.6% were IBS-M, in their study, which included students of medical college.

In our study of IBS patients, mean IBS-SSS score was 215.2 ± 65.6. Parker et al[108] found mean score of IBS-SSS to be 236.6 ± 85.4 in IBS patients. Mean IBS-SSS was 279.8 in another study.[109] Mean IBS-QOL score of our study was 66.7 ± 15.0. Passos et al[109] found IBS-QOL score to be 83.3. Magdalena et al[110] found mean IBS-QOL score to be 61.62 ± 19.88, which was significantly higher than healthy controls. Björkman et al[111] found that some sub-scores of IBS-SSS like (extra-colonic score) and sub-scores of IBS-QOL (domains mental health, sleep, social role, or physical role), were similar in men and women. Our study also found no difference between men and women for overall score for IBS-SSS and IBS-QOL.

In our study, prevalence of anxiety and depression were 84.8% and 67.0% respectively. Haug et al[112] also found a strong relationship between gastrointestinal symptoms, anxiety and depression. Banerjee et al[67] found the prevalence of anxiety to be 44% and that of depression to be 84%. There is mixed data regarding distribution of anxiety and depression in males and females in IBS patients. Björkman et al[111] observed that anxiety was significantly higher in women as compared to men. Other authors also confirmed significantly higher depression rates in IBS women compared to men.[16,113] The gender differences observed may be due to the cultural background and lower number of men seeking medical help.[114] In addition, women are more exposed to stressful events and risk factors for depression during their life.[113] But there are studies in IBS which found no significant difference between men & women, for anxiety & depression.[115,116] In our study also, there was similar distribution of anxiety and depression among male and female IBS patients.

Our study evaluated prevalence of SIBO in IBS patients, in present time, in north India. Of the 112 IBS patients we studied, SIBO was present in 32.1%. In the previous studies, prevalence of SIBO has ranged from 11.1% to 46 % in IBS patients of western countries[85,117] and from 8.5% to 32.9% in studies done in IBS patients in India.[118,119] Rana et al[91] found prevalence of SIBO in IBS to be 11.1 % based on GHBT. Sachdeva et al[92] found it to be 23.7%. Grover et al[119] also studied SIBO in IBS patients, in which it was found to be 32.9%. Another study by Carrara et al[120] reported it to be 43%. SIBO was found to be as high as 45.8% in Italian population, in study done by Cuoco et al.[84]

While comparing various subtypes of IBS, SIBO was found to be significantly higher in diarrhea predominant IBS in our study (p = 0.036). Our results were in accordance with the previously done study done by Sachdeva et al[92], in which patients with IBS-D more often had SIBO on GHBT compared with non-diarrheal IBS (p =0.02).[92] Ghoshal et al[121] also found that patients with SIBO more often had diarrhea, higher stool frequency, and looser form of stools. But contrary to these, Grover et al[119] found no significant difference between IBS sub-types in SIBO positive or SIBO negative IBS patients. The pattern of predominance of SIBO (42%) in IBS-D subtype patients is evidenced in western literature also, which was significantly higher as compared to other subtypes, as suggested by Lupascu et al.[83] In a Romanian study, done by Moraru et al[87] SIBO was present in nearly half of the IBS-D patients (45.7%), thus diarrhea was predictive of SIBO.

We found relatively higher prevalence of SIBO as compared to other studies done in India, probably because of enrolment bias or higher proportion of diarrhea predominant IBS patients in our community. Factor contributing to higher proportion of diarrhea predominant IBS and indirectly to higher prevalence of SIBO, could be different composition of microbiota in small intestine of our study population. Further studies are required to evaluate the microbiota to confirm this finding. Wu et al[90] studied microbiota of IBS patients and found that SIBO was present in 51.7% of the IBS-D patients, and there was evidence of increased

abundance of *Prevotella* species, in their study population. Prevalence of SIBO in IBS-D patients in our study was 38.7%.

When SIBO was compared between different age groups, no significant difference was found (p = 0.09), i.e., SIBO was uniformly distributed among various age groups. There was also no significant difference between presence or absence of SIBO, based on gender of patients (p = 0.234) in our study. Similar results were also found in 2014 by Abbasi and colleagues[88], who found prevalence of SIBO to be 37%, with no difference based on age or gender of patients. Our results were in accordance with the results of study done by Ghoshal et al[121] in 2014 & Wu et al[90] in 2019, in which both the male and female IBS patients had proportional distribution of SIBO, and its prevalence was not affected by age, although the latter study included only diarrhea predominant IBS. Rana et al[91] also concluded in study of IBS patients that prevalence of SIBO in male and female patients was not significantly different. Another Indian study by Sachdeva and colleagues[92] in 2011 suggested that female IBS patients had significantly higher prevalence of SIBO, but age was not a significant variable in their study. The only limitation of their study was sample size of 57. Chu et al[89] found that SIBO was present in 39% of the IBS patients, on comparing age and gender between the SIBO positive and SIBO negative group, no significant difference was found, similar as in our study. Erdogan et al[122] found that mean age was significantly higher in patients who were GBT positive. The study evaluated SIBO in general population, not in IBS patients. Age might be an important factor in SIBO in general, but not for SIBO in IBS population, where other factors like IBS subtype, presence of sub-clinical intestinal inflammation play an important role. Our results regarding effect of BMI on SIBO in IBS patients, were in accordance with results of Chu et al[89] who found no significant difference of BMI between two groups.

We found that IBS-SSS, i.e., Symptom severity score was significantly higher in patients who tested positive for SIBO (p<0.001). Our results were in accordance with study done by Wu et al[90] who also found mean

IBS-SSS in SIBO-positive subgroup of IBS patients was significantly higher than SIBO-negative subgroup. Our results for IBS-QOL were also in accordance with Wu et al[90], who found that SIBO positive patients suffered from unfavourable life quality when compared with SIBO negative counterpart.

Grover et al found that IBS patients had significantly higher IBS-SSS score than healthy controls. However, there were no significant differences between SIBO positive and SIBO negative IBS patients on the IBS-SSS. But one limitation of that study was that they used sucrose as substrate for HBT. Although, anxiety and depression were present in higher number of IBS patients who were SIBO positive as compared to those who were SIBO negative, but the difference was not significant ($p = 0.575$) and ($p = 0.213$), respectively. Chu et al[89] found IBS patients as compared to controls had significantly higher scores of anxiety and depression, but the comparison between SIBO positive and SIBO negative patients exhibited similar scores for anxiety and depression. Wool et al[123] also found similar rate of anxiety and depression in IBS patients, regardless of them being positive or negative for SIBO.

Mean fecal calprotectin in IBS patients of our study was 41.6 ± 27.2 μg/g. 72.3% (81/112) of IBS patients had fecal calprotectin level between 0-50 μg/g; 23.2% (26/112) had fecal calprotectin between 50-100 μg/g; 1.8% (2/112) and 2.7% (3/112) patients had fecal calprotectin between 100-150 & 150-200, respectively. Maximum value of fecal calprotectin in our study was 167 μg/g. Burri et al[37] found faecal calprotectin level of 50 μg/g of faeces discriminated organic from non-organic intestinal disease with 84.4% sensitivity and 94.5% specificity. They also showed that higher cut-off (100 μg/g) showed a higher positive predictive value but a lower negative predictive value and sensitivity than the lower cut-off value (50 μg/g). Fecal calprotectin in their study ranged from 10-210 μg/g. Another study by Sydora et al[36] found that fecal calprotectin in IBS patients ranged from 16 – 222 μg/g. In our study we took cut-off value of fecal calprotectin as 50 μg/g to compare characteristics between

patients with normal fecal calprotectin and raised fecal calprotectin, with the idea that raised fecal calprotectin suggests evidence of sub-clinical intestinal inflammation in IBS patients. Melchior et al[38] also discussed characteristics of IBS patients based on similar cut-off as our study. They found that mean fecal calprotectin was 90.5 ± 180 μg/g and a subgroup of 34 patients (36.6%) had levels higher than 50 μg/g. No patient with abnormal levels had diverticular disease or colorectal adenoma or was taking medications (mainly nonsteroidal anti-inflammatory drugs) known to increase the fecal calprotectin level. No patient developed IBD for 1 year follow-up. Grad et al[124] also proved that fecal calprotectin was significantly raised in IBS patients as compared to healthy controls. Melchior et al[38] found that IBS-D was the most common subtype of patients with raised fecal calprotectin. Similar results were seen in our study, in which fecal calprotectin was raised in 27.7% of IBS patients and IBS-D subtype had significantly higher proportion of patients with raised fecal calprotectin, as compared to other subtypes.

Our results were in accordance with study done by Melchior et al[38], who found that IBS patients with raised fecal calprotectin were older as compared to those with normal levels, but the difference was not significant. There was similar distribution of raised fecal calprotectin among males and females. Melchior et al[38] found that mean duration of IBS symptoms was not significant among two groups of raised vs normal fecal calprotectin. Our results were contradictory and found that IBS patients with longer duration of disease had higher mean fecal calprotectin level. Further research is needed with higher number of patients to confirm this relationship. Our results regarding effects of BMI on intestinal inflammation were in accordance with results of Melchior et al, who found no significant difference between the BMI of IBS patients with raised vs normal fecal calprotectin. Melchior et al[38] found that although mean IBS-SSS was higher in IBS patients with raised fecal calprotectin as compared to those with normal levels, the difference was not statistically significant. Intriguingly, our study found the difference to be significant ($p < 0.001$). This states that IBS patients with raised fecal

calprotectin in our study were having higher severity of symptoms. Melchior et al[38] found quality of life score calculated by GIQLI was not different among the patients with raised fecal calprotectin from those with normal level, but our study found the difference to be significant ($p < 0.001$). Further research is needed to compare larger population to find association of intestinal inflammation with severity of symptoms and quality of life. Melchior et al[38] found that there was no significant difference of mean anxiety and depression scores in IBS patients with raised fecal calprotectin from normal fecal calprotectin. Similar results were seen in our study, with prevalence of anxiety and depression was comparable between two groups.

Our study found that out of 36 patients who had SIBO, fecal calprotectin was raised in 29 which translates to 80.6%. Significantly higher number of SIBO positive IBS patients had raised fecal calprotectin levels as compared to those with SIBO negative ($p = 0.001$). Our results were concordant with the previously done study by David et al.[101] Thus, SIBO is associated with sub-clinical intestinal inflammation.

IBS patients who were positive for SIBO or were having raised fecal calprotectin or both, underwent colonoscopy to rule out organic disease like IBD. None of the patients who underwent colonoscopy was found to have organic disease. Such patients received rifaximin by the primary treating team in dose of 550mg three times a day for 2 weeks.[94] After 12 weeks of initial enrolment, all IBS patients were re-evaluated for assessment of change in IBS-SSS and IBS-QOL scores.

On follow-up, in patients who had SIBO positive at baseline (n=36), IBS-SSS and IBS-QOL improved in 86.1% (31/36) and in 83.3% (30/36), respectively. ($p = 0.067$ and $p = 0.003$, respectively). In patients who had raised fecal calprotectin at baseline (n=31), IBS-SSS and IBS-QOL improved in 90.3% (28/31) and in 87.1% (27/31). ($p = 0.027$ and $p = 0.001$, respectively). Meyrat et al[75] also found significant improvement of symptoms in IBS patients with SIBO after 2 weeks treatment with rifaximin. Majewski et al[85] found that symptoms score improved in

87.5% IBS patients with SIBO after treatment with rifaximin. Rezaie et al found the effectiveness of rifaximin to be 76.5% in IBS patients who were HBT positive.[94]

Thus, improvement in severity of symptoms and quality of life was more in those IBS patients who were having SIBO or raised fecal calprotectin and were treated with rifaximin. Diagnosis and treatment of SIBO and intestinal inflammation in IBS patients can lead to better control of symptoms and improvement in quality of life.

Our study had few limitations. We may have overestimated the prevalence of SIBO in IBS because being a tertiary care centre, we receive patients who have severe and refractory disease. There were no healthy controls in our study to compare SIBO and fecal calprotectin with IBS patients. This was a non-interventional study. Follow-up assessment was done only on the basis of symptom severity and quality of life scores. Resolution of SIBO using GBT was not confirmed on follow up. Long-term follow-up to assess recurrence of SIBO was not done.

The strength of our study was that it was first of its kind in IBS patients in north India. We assessed correlation of SIBO with fecal calprotectin in IBS patients, data of which is scarce. We also evaluated effect of SIBO and intestinal inflammation in IBS patients on clinical parameters like severity of symptoms and quality of life.

SUMMARY AND CONCLUSION

Irritable bowel syndrome is a gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits in the absence of any organic cause. Total 112 IBS patients diagnosed according to ROME IV criteria were included in the study.

- Mean age of the patients was 35.8 ± 11.9 years, mean duration of symptoms was 2.7 ± 2.5 years and mean BMI was 24.7 ± 4.1 kg/m^2.
- 33.9% (38/112) of the patients were females and 66.1% (74/112) of the patients were males.
- Majority of the patients i.e., 33% of total IBS patients were those with duration less than 1 year (37/112), followed by 1-2 years (33/112), followed by >3 years (25/112), and lastly those with duration 2-3 years (17/112).
- On classifying IBS patients according to BMI, majority of the patients, i.e., 33.9% (38/112) belonged to 'obese – I' class. If we combine obese - I and obese – II classes, IBS patients can be categorized into obese vs non-obese, i.e., 44.6% (50/112) vs 55.4% (62/112), respectively.
- Among the three sub-types of IBS, i.e., constipation predominant IBS (IBS-C), diarrhea predominant IBS (IBS-D) and mixed type IBS (IBS-M); most common sub-type of IBS in the present study was IBS-D (71.4%), followed by IBS-C (18.8%) and lastly IBS-M (9.8%).
- In IBS patients, mean IBS-SSS score, that indicates severity of symptoms was found to be 215.2 ± 66.6 and mean IBS-QOL score, which estimates IBS specific quality of life was found to be 66.7 ± 15.0.
- Duration of disease had significant impact on the IBS-SSS and IBS-QOL score ($p < 0.002$).
- There was no significant difference of clinical parameters between obese and non-obese patients. Also, there was no significant difference between males and females.

- **SIBO** was present in 32.1% (36/112) of the IBS patients. While comparing the prevalence of SIBO among three sub-types of IBS, IBS-D had the highest prevalence. And the difference was statistically significant (p 0.036).
- SIBO was uniformly distributed among all age groups. SIBO was found to be higher in males as compared to females, but the difference was not significant (p = 0.23). IBS patients who had longer duration of disease, were significantly more likely to have SIBO. (p = 0.001). Higher BMI was associated with higher prevalence of SIBO, but the difference was not significant (p = 0.303).
- IBS-SSS was significantly higher in patients who tested positive for SIBO (p<0.001). IBS-QOL, which measures quality of life in IBS patients (higher is better), was significantly lower in SIBO positive patients as compared to SIBO negative patients (p<0.001).
- Mean **fecal calprotectin** level in the study population was 41.6 ± 27.2 μg/g, and its cut-off value to define raised vs normal fecal calprotectin level was taken as 50 μg/g. Raised fecal calprotectin level (intestinal inflammation) was found in 27.7 % (31/112) of the IBS patients.
- On comparing the presence of intestinal inflammation among three sub-types of IBS, IBS-D had the highest prevalence. And the difference was statistically significant (p 0.030).
- Patients with longer duration of disease, tend to have significantly higher prevalence of intestinal inflammation (p = 0.014).
- Prevalence of intestinal inflammation was similar in all age groups (p = 0.09). It was more prevalent in males than females, but the difference was not statistically significant (p = 0.268). Although proportion of patients with raised fecal calprotectin was higher in obese, as compared to non-obese patients, the difference was not statistically significant (p = 0.538).

- IBS-SSS was significantly higher in patients who had raised fecal calprotectin level ($p < 0.001$). IBS-QOL, which measures quality of life in IBS patients (higher is better), was significantly lower in patients who had raised fecal calprotectin level ($p < 0.001$).
- Out of 36 patients who had SIBO, fecal calprotectin was raised in 29, which translates to 80.6%. Significantly higher number of SIBO positive IBS patients had raised fecal calprotectin levels as compared to those with SIBO negative. ($p = 0.001$)
- IBS patients who were positive for SIBO or were having raised fecal calprotectin or both, underwent colonoscopy to rule out organic disease like IBD. None of these patients were found to have organic disease. These patients received rifaximin by the primary treating team in dose of 550mg three times a day for 2 weeks.
- At 12 weeks follow-up, in patients who had SIBO at baseline (n=36), IBS-SSS and IBS-QOL improved in 86.1% (31/36) and in 83.3% (30/36). ($p = 0.067$ and $p = 0.003$, respectively).
- At 12 weeks follow-up, in patients who had raised fecal calprotectin at baseline (n=31), IBS-SSS and IBS-QOL improved in 90.3% (28/31) and in 87.1% (27/31). ($p = 0.027$ and $p = 0.001$, respectively).

To conclude, our study found that significantly higher number of SIBO positive IBS patients had raised fecal calprotectin levels as compared to those with SIBO negative, ($p = 0.001$). Mean IBS-SSS and IBS-QOL were significantly higher in those IBS patients who had SIBO or raised fecal calprotectin. The clinically meaningful improvement in severity of symptoms and quality of life was more in IBS patients who had SIBO or raised fecal calprotectin and got treatment. Thus, our study highlights the importance of assessment and treatment of SIBO and intestinal inflammation in patients clinically diagnosed with IBS, as treatment of these lead to symptomatic improvement.

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KEY TO MASTER CHART

BMI	Body Mass Index
CRP	C-Reactive Protein
F	Females
FC	Fecal Calprotectin
FU	Follow Up
HAMA	Hamilton Anxiety Rating Scale
HAMD	Hamilton Depression Rating Scale
Hb	Hemoglobin
HBT	Hydrogen Breath Test
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome with Constipation
IBS-D	Irritable Bowel Syndrome with Diarrhea
IBS-M	Mixed type Irritable Bowel Syndrome
IBS-QOL	Irritable Bowel Syndrome Quality of Life
IBS-SSS	Irritable Bowel Syndrome Severity Scoring System
M	Males
MRD	Medical Records Department
SIBO	Small Intestinal Bacterial Overgrowth
TSH	Thyroid Stimulating Hormone
TTG	Tissue Trans-Glutaminase

Serial number	Mrd number	Age	Sex	IBS type D/C/M	Duration (years)	BMI (Kg/m2)	Fecal calprotectin (µg/g)	SIBO (GHBT)	IBS-SSS (Baseline)	IBS-QOL (Out of 170) (Baseline)	IBS-QOL (Baseline)	HAM-A	HAM-D	IBS-SSS (FU)	IBS-SSS (Change)	IBS-QOL (out of 170)(FU)	IBS-QOL (FU)	IBS-QOL (Change - Out of 170)	Hb	MCV	Creat.	ALT	CRP(Q)	TTG	TSH
1	1132909	20	M	M	0.5	29	<25	Negative	220	89	59.56	23	13	180	40	68	75.00	21.0	15.3	94.1	0.8	29	1	0.3	1.95
2	1005316	25	M	C	1	20.1	<25	Negative	190	58	82.35	8	6	150	40	53	86.03	5.0	16.1	93	0.5	13	0.28	0.3	1.95
3	1157146	43	M	D	1	24.6	30	Negative	260	86	61.76	12	11	180	80	67	75.74	19.0	14.2	88.8	0.5	18	1.2	0.3	1.5
4	1126709	30	M	D	3	23.1	<25	Negative	260	85	62.50	11	9	140	120	79	66.91	6.0	14.9	82.7	0.9	30	2.5	0.1	1.8
5	1141821	45	F	C	2	30.6	28	Negative	210	88	60.29	21	6	110	100	66	76.47	22.0	11.7	88	1	23	1.3	1	3.6
6	1170034	22	M	D	2	27	32	Negative	220	77	68.38	12	4	130	90	56	83.82	21.0	14.5	90	1.1	42	1.5	1.2	3
7	1064944	26	M	D	4	29.3	51	Positive	280	92	57.35	18	13	230	50	92	57.35	0.0	15.8	87.1	1.1	114	1.63	1.5	2.68
8	73290	23	M	D	2	18.4	60	Positive	240	49	88.97	3	3	100	140	44	92.65	5.0	15.2	89.4	0.9	19	3.6	1.6	2.3
9	1159555	42	M	C	6	32.7	<25	Negative	140	50	88.24	11	8	90	50	45	91.91	5.0	11.5	90	0.6	32	3	0.3	3.2
10	767956	51	F	M	2	22.6	51	Positive	240	85	62.50	16	11	150	90	73	71.32	12.0	10.2	64.2	0.7	21	1.2	1.2	3.2
11	1133491	20	F	M	1	14.9	<25	Negative	110	55	84.56	5	4	90	20	46	91.18	9.0	13.1	92.4	0.6	41	3.2	0.4	1.39
12	1138746	30	M	D	5	35.2	56	Positive	330	97	53.68	16	7	210	120	91	58.09	6.0	12.2	82	1.2	36	4.2	1.8	4
13	1126168	30	F	C	0.5	24.2	6.9	Negative	150	60	80.88	8	5	60	90	46	91.18	14.0	13.2	90	1.1	32	1.5	1.2	2.5
14	1157514	48	M	D	1	20.2	36	Negative	250	63	78.68	18	5	160	90	61	80.15	2.0	10.5	68.5	0.9	17	3	2	1.1
15	1124882	58	M	D	9	26.9	64	Positive	160	71	72.79	6	6	60	100	54	85.29	17.0	12.2	90	0.8	32	1.8	1.4	2.9
16	42151	44	M	D	1.5	23.8	<25	Negative	160	64	77.94	11	8	140	20	59	81.62	5.0	14.4	82.8	0.8	22	1.8	1.3	1.76
17	497363	27	M	D	0.75	29.2	56	Positive	310	91	58.09	21	8	150	160	68	75.00	23.0	13.4	88	0.8	33	5.8	2.2	2.5
18	1108948	26	M	D	2	28.6	<25	Negative	190	84	63.24	10	7	160	30	60	80.88	24.0	14.2	85.4	0.9	35	2.2	1.8	3.2
19	1157225	48	M	C	0.5	26	<25	Negative	120	67	75.74	8	6	80	40	47	90.44	20.0	13.5	88	1	35	3	1.1	3.3
20	1077353	19	M	C	0.6	35.4	<25	Negative	180	62	79.41	11	7	80	100	51	87.50	11.0	13.1	88	0.7	28	1.4	2.1	3.2
21	1069733	25	F	D	1	20.7	42	Negative	240	80	66.18	11	8	140	100	67	75.74	13.0	13.2	87	0.8	14	3	1.2	3.5
22	963291	26	F	D	2	17.7	56	Positive	260	92	57.35	22	9	150	110	65	77.21	27.0	11.9	80.6	0.9	32	1.6	0.7	1.5
23	465601	52	F	D	3	25	32	Negative	190	80	66.18	13	7	120	70	56	83.82	24.0	14.2	93	1.1	22	3.3	1.2	3.5
24	872230	23	M	D	3	22.5	35	Negative	180	73	71.32	14	9	120	60	54	85.29	19.0	13.5	88	1.1	26	2.5	3.2	1.5
25	1095945	30	M	D	2	29.5	57	Positive	290	78	67.65	13	13	140	150	56	83.82	22.0	13.2	83	0.8	97	0.2	0.3	2.9
26	313198	23	M	D	0.5	20.1	40	Negative	280	81	65.44	12	12	180	100	64	77.94	17.0	13.8	72	0.6	16	0.5	0.5	1.1
27	1123788	36	F	C	3	30.4	55	Positive	290	89	59.56	20	12	200	90	65	77.21	24.0	12.7	84.5	0.9	22	2.5	2	3.4
28	616763	46	M	M	1	27	<25	Negative	160	68	75.00	17	11	100	60	46	91.18	22.0	14.1	82	0.9	44	1.7	1.3	2.2
29	1146954	40	M	D	2	26	55	Positive	280	92	57.35	19	9	200	80	62	79.41	30.0	14	89	0.8	39	3.2	1.2	3.3
30	1056455	23	M	D	2	23.5	44	Positive	320	89	59.56	19	12	230	90	63	78.68	26.0	14.7	88.9	0.8	72	4.5	2.1	2.1
31	838411	30	M	D	1	33.8	26	Negative	190	66	76.47	18	10	100	90	50	88.24	16.0	13.5	87	0.7	62	1.2	2.1	1.1
32	1117329	29	F	D	1.5	24.8	62	Positive	310	146	17.65	9	6	200	110	95	55.15	51.0	13.2	85	1.1	36	4.8	2.2	3.2
33	1127240	57	M	D	10	23.8	50	Positive	260	98	52.94	18	11	160	100	70	73.53	28.0	13.1	80.7	0.7	18	6.8	0.4	2.5
34	972246	38	M	D	1.5	24.2	43	Negative	220	78	67.65	17	14	170	50	60	80.88	18.0	12.2	87	0.8	22	2.8	2.1	1.1
35	1140675	28	M	D	0.5	25.3	30	Negative	160	55	84.56	12	10	100	60	48	89.71	7.0	13.3	81.2	0.6	26	3.3	0.3	2.2
36	1114010	19	F	D	1	18.7	43	Positive	190	70	73.53	19	16	160	30	56	83.82	14.0	15.2	93.3	0.6	21	0.5	0.3	3.5
37	1114875	35	M	D	6	19.75	52	Positive	320	80	66.18	18	11	150	170	60	80.88	20.0	16	92.2	1.1	18	3.6	3	1.1
38	1022164	39	M	D	4	27.8	102	Positive	330	102	50.00	19	18	220	110	80	66.18	22.0	15.1	84	0.6	27	2.1	0.1	4.2
39	1120973	26	M	C	2	31.3	<25	Negative	150	52	86.76	12	12	90	60	44	92.65	8.0	13.2	87	0.4	43	0.8	2.1	2.2
40	1141303	31	F	D	1.5	17.6	92	Positive	320	102	50.00	18	16	200	120	80	66.18	22.0	14.2	84.5	0.5	36	6.4	1.1	2.2
41	498740	59	M	D	10	28	37	Negative	260	87	61.03	18	5	190	70	65	77.21	22.0	12.6	79	0.6	32	3.3	2.1	2.3
42	1035005	42	F	D	2	30.6	162	Positive	260	82	64.71	7	6	180	80	65	77.21	17.0	13.5	84	1.2	40	4.5	0.3	3.4
43	1083467	25	M	C	2	32	39	Negative	180	64	77.94	9	6	100	80	61	80.15	3.0	14.7	90.7	1.1	42	2.5	0.5	2.2
44	1120901	23	M	D	0.5	24.9	<25	Negative	150	66	76.47	12	4	120	30	48	89.71	18.0	13.2	87.3	0.6	29	1.2	2.1	3.4
45	1099194	22	M	M	0.5	23.1	<25	Negative	140	56	83.82	12	7	90	50	43	93.38	13.0	12.5	80.3	0.4	22	2.3	0.5	2.2
46	467148	54	M	D	5	23.1	30	Negative	180	65	77.21	18	6	120	60	54	85.29	11.0	16.5	83	0.6	33	3	6	3.6
47	1139273	19	M	D	1	27	30	Negative	190	76	69.12	12	10	80	110	54	85.29	22.0	16	92	0.8	35	0.6	0.6	2.1
48	1188234	33	M	C	0.5	24.9	<25	Negative	150	56	83.82	10	9	90	60	38	97.06	18.0	14.6	89.6	0.5	33	0.9	0.5	2.1
49	1126691	48	F	D	1	21.9	<25	Negative	140	59	81.62	17	19	110	30	45	91.91	14.0	12.5	83	0.8	42	1.2	1.3	2.2
50	977378	27	M	D	2	18.9	55	Positive	340	122	35.29	19	9	210	130	60	80.88	62.0	11.2	90	1	23	4.4	2.1	3.3
51	857572	44	M	C	1	25.3	<25	Negative	120	40	95.59	11	10	70	50	34	100.00	6.0	13.3	89	1.1	34	2.1	2.2	3.7
52	1120016	35	M	D	3	28.2	57	Positive	290	92	57.35	21	11	210	80	66	76.47	26.0	15	90.1	0.9	33	2.5	2.1	1.5
53	1111142	28	F	D	1.5	14.8	<25	Negative	170	66	76.47	17	16	110	60	54	85.29	12.0	10.2	80.6	0.8	25	2.1	2.3	1.4
54	1125174	24	F	D	5	25.8	56	Positive	320	103	49.26	21	11	220	100	87	61.03	16.0	13.2	87.8	1.1	32	5.5	3.5	4.3
55	941691	55	F	D	6	25	30	Negative	180	77	68.38	13	3	90	90	56	83.82	21.0	13.7	88.9	0.6	33	2.1	2.2	2.1
56	1123426	42	M	D	0.5	24.2	<25	Negative	150	67	75.74	11	3	110	40	53	86.03	14.0	12.5	82.1	0.7	46	0.8	1.5	2.9

Serial number	Mrd number	Age	Sex	IBS type D/C/M	Duration (years)	BMI (Kg/m2)	Fecal calprotectin (µg/g)	SIBO (GHBT)	IBS-SSS (Baseline)	IBS-QOL (Out of 170) (Baseline)	IBS-QOL (Baseline)	HAM-A	HAM-D	IBS-SSS (FU)	IBS-SSS (Change)	IBS-QOL (out of 170)(FU)	IBS-QOL (FU)	IBS-QOL (Change - Out of 170)	Hb	MCV	Creat.	ALT	CRP(Q)	TTG	TSH
57	1125163	23	M	D	1	24.4	<25	Negative	170	59	81.62	18	5	100	70	49	88.97	10.0	14.9	78.2	0.9	45	1.1	2.3	2.6
58	265755	29	M	D	2	24.7	<25	Negative	160	60	80.88	18	3	90	70	52	86.76	8.0	14	88	0.8	30	5.1	2.2	2.7
59	1054868	21	M	D	1	19.9	30	Negative	180	58	82.35	5	4	140	40	45	91.91	13.0	14.1	88.3	1	36	1.5	0.4	2.3
60	931891	42	F	D	2	17.4	158	Positive	320	118	38.24	34	15	210	110	68	75.00	50.0	12.3	89.8	1.1	10	5.9	1.2	2.3
61	1114836	41	F	D	7	24.6	52	Positive	200	127	31.62	18	14	90	110	63	78.68	64.0	11.7	79	0.8	36	4.3	0.9	2.5
62	860012	51	F	D	3	28.1	56	Positive	220	83	63.97	26	18	190	30	53	86.03	30.0	11.1	90	0.6	20	4.1	1.4	2.1
63	1116664	30	M	C	1	23.5	27	Negative	180	60	80.88	11	11	100	80	46	91.18	14.0	13.3	89	0.6	12	1.6	2.1	2.3
64	979626	29	M	D	2.5	23.8	51	Negative	260	88	60.29	18	11	110	150	67	75.74	21.0	14.1	85	0.7	23	4.1	0.7	2.1
65	1140566	36	M	D	1	26.6	<25	Negative	170	84	63.24	16	14	130	40	60	80.88	24.0	14.7	88	0.7	19	2.1	2.2	3.4
66	261698	28	F	C	3	18.4	22	Negative	180	60	80.88	23	11	110	70	46	91.18	14.0	13.2	89	0.5	33	3.4	2.1	2.2
67	255792	59	F	C	10	29	43	Negative	220	93	56.62	22	17	100	120	70	73.53	23.0	12.5	81	0.5	32	3.3	1.2	2.2
68	1137861	52	M	D	3	22.4	<25	Negative	150	70	73.53	11	10	80	70	50	88.24	20.0	14.4	86.7	0.7	31	2.7	2.2	4.6
69	1137076	30	M	D	1.5	23.5	<25	Negative	160	75	69.85	18	11	90	70	60	80.88	15.0	15.9	89.2	0.7	28	1.1	0.4	3.3
70	1129453	53	M	D	4	30	167	Positive	350	132	27.94	22	21	220	130	80	66.18	52.0	14	83.6	0.6	52	6.5	2.2	1.8
71	1001764	48	F	C	2	25.3	33	Positive	190	87	61.03	18	14	170	20	69	74.26	18.0	9.1	78.5	0.8	33	3.1	1.1	1.5
72	1114861	55	M	C	7	28.4	42	Negative	200	83	63.97	9	6	100	100	54	85.29	29.0	13.2	82.5	1	32	2.2	2.1	1.8
73	1022833	25	M	D	2	28	35	Negative	180	78	67.65	19	14	120	60	60	80.88	18.0	12.6	80.2	1.1	26	5.4	2.1	2.2
74	1060389	27	M	D	3	24.8	48	Negative	290	88	60.29	23	18	170	120	70	73.53	18.0	14.5	90.5	0.9	25	3.4	0.2	1.1
75	1120340	35	F	D	3	24.2	53	Positive	270	84	63.24	27	5	190	80	62	79.41	22.0	12.3	83.2	0.6	33	4.1	2.2	1.3
76	1124581	24	M	C	2	25.4	30	Negative	170	78	67.65	18	15	130	40	67	75.74	11.0	14.4	87.9	0.6	33	1.2	0.7	3.2
77	1141320	33	M	D	3	20.7	47	Negative	260	93	56.62	22	18	120	140	74	70.59	19.0	14	88	0.5	21	4.4	1.4	2.3
78	749400	25	M	C	3	21.6	30	Negative	200	90	58.82	17	13	130	70	65	77.21	25.0	12.1	87.7	0.5	34	0.9	1.1	2.3
79	1125265	58	M	D	10	24.2	66	Positive	360	97	53.68	18	16	210	150	50	88.24	47.0	13.2	86.7	0.4	33	5	1.6	2.3
80	1141334	45	F	D	1	25.4	30	Negative	210	82	64.71	18	11	130	80	69	74.26	13.0	12.8	91	0.8	31	2.1	0.5	2.1
81	1161651	33	F	M	6	16	51	Negative	340	112	42.65	25	21	210	130	80	66.18	32.0	11.8	80	0.9	33	3.2	0.7	2.6
82	1114867	59	F	M	12	33.7	<25	Negative	150	51	87.50	4	3	90	60	50	88.24	1.0	13	95	0.7	36	1.3	2.2	2.2
83	1139075	23	F	D	1.5	24.2	<25	Negative	160	59	81.62	9	10	90	70	39	96.32	20.0	12.9	89.9	0.9	21	0.24	0.7	2.8
84	1119098	22	M	D	0.5	23.4	42	Negative	130	52	86.76	6	5	80	50	40	95.59	12.0	16	96.3	1	40	1.3	0.4	2.1
85	1110762	44	M	D	5	23.8	<25	Negative	120	52	86.76	7	10	80	40	41	94.85	11.0	12.8	85.6	1.1	27	2.4	1.1	2.4
86	1126078	23	M	C	1.5	23.7	<25	Negative	150	61	80.15	8	11	70	80	41	94.85	20.0	15.8	91.5	0.7	22	0.5	2.1	1.6
87	1117187	21	M	D	4	18.6	55	Positive	300	128	30.88	16	6	210	90	84	63.24	44.0	14.5	84	0.6	15	4.1	0.4	2.4
88	1166503	36	M	C	1	22.1	<25	Negative	140	67	75.74	18	11	90	50	45	91.91	22.0	15.5	101	0.7	36	1.2	0.8	1.1
89	1103809	26	M	M	1	20.7	<25	Negative	150	75	69.85	10	9	130	20	65	77.21	10.0	14.3	89.8	0.9	21	1	1.1	2.1
90	1082533	53	M	M	5	25.5	<25	Positive	150	58	82.35	4	4	90	60	45	91.91	13.0	15.2	87.5	0.8	20	1.4	2.2	1.2
91	1126205	31	F	D	1	22.5	<25	Negative	160	66	76.47	8	9	80	80	45	91.91	21.0	14.5	88.3	0.5	31	2.1	2.2	3.2
92	756461	37	F	D	0.5	22	<25	Negative	130	51	87.50	5	8	70	60	39	96.32	12.0	11.5	80	0.8	21	1.1	2.1	1.1
93	1097471	37	M	D	1	24.5	<25	Negative	120	49	88.97	8	9	60	60	41	94.85	8.0	13.4	81	0.7	22	2.1	2.2	3.1
94	1165677	45	F	D	3	30.5	55	Positive	280	112	42.65	19	21	170	110	78	67.65	34.0	10.5	86.9	0.6	24	5.8	0.5	5.2
95	1146936	38	F	D	2	23.1	30	Negative	210	89	59.56	19	18	180	30	67	75.74	22.0	11.5	88	0.6	35	0.13	1.8	2.1
96	1124038	26	M	D	2	26.6	39	Negative	190	91	58.09	18	20	110	80	70	73.53	21.0	16.1	91.7	0.5	87	2.1	0.3	2.1
97	1138775	32	M	M	3	27.8	147	Positive	330	112	42.65	25	21	210	120	78	67.65	34.0	13.3	86	1.1	73	5.2	2.1	1
98	1126692	47	F	D	2	24.9	40	Negative	270	98	52.94	20	18	170	100	70	73.53	28.0	12.4	81	1	32	2.1	2.2	1.1
99	1133887	19	F	D	0.5	19.4	<25	Negative	160	55	84.56	8	10	90	70	41	94.85	14.0	10.8	88	1.1	16	0.7	1.1	2.2
100	1033563	41	F	D	2	25.7	43	Negative	260	82	64.71	18	16	170	90	58	82.35	24.0	11.2	89	0.6	21	2.2	0.9	2.2
101	688828	46	M	C	1.5	23.5	<25	Negative	150	65	77.21	18	21	100	50	54	85.29	11.0	14.4	100.3	0.9	33	1.2	0.6	1.1
102	1036854	47	F	D	1	25.7	<25	Negative	140	77	68.38	19	15	100	40	45	91.91	32.0	12.1	79.5	0.79	22	1.4	2.1	0.9
103	1133497	50	F	D	2.5	20.4	<25	Negative	170	67	75.74	10	10	90	80	47	90.44	20.0	13.3	84	0.6	21	3.2	1.1	2.3
104	1126730	45	M	D	1	27.5	33	Negative	270	87	61.03	21	19	160	110	67	75.74	20.0	14.4	80	0.5	23	2.2	1.5	2.2
105	1110405	60	M	D	11	27	48	Positive	320	129	30.15	27	20	200	120	89	59.56	40.0	13.6	91	0.7	21	1.1	1.1	0.8
106	1134110	55	F	D	7	23.4	13	Negative	160	51	87.50	10	7	100	60	39	96.32	12.0	12	88.1	0.6	29	2.6	1.1	0.7
107	1120545	58	M	D	9	26.7	57	Positive	290	98	52.94	24	21	200	90	76	69.12	22.0	14.4	87.4	0.9	25	9.7	0.4	2.2
108	1069086	29	F	D	3.5	28.7	48	Positive	280	88	60.29	23	19	180	100	69	74.26	19.0	13.4	90	0.4	28	5.1	1.2	1.8
109	926831	25	M	M	2	19.6	30	Negative	190	79	66.91	18	12	110	80	60	80.88	19.0	13	88	0.8	22	0.3	2.1	0.9
110	1154243	36	M	D	3	28.1	51	Positive	240	102	50.00	17	11	190	50	64	77.94	38.0	13.1	87	0.7	32	5.1	1.2	0.9
111	1119268	32	M	D	1	21.5	<25	Negative	190	87	61.03	18	19	80	110	78	67.65	9.0	14.4	91.3	0.9	22	1.8	0.4	1.6
112	1143929	23	M	D	1.5	25.1	26	Negative	180	79	66.91	12	10	100	80	50	88.24	29.0	12.2	98.7	1	38	2.7	1.1	1.1

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The Principal,
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Subject: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 22.01.2021 has approved the plan of thesis of **Dr Aakash Aggarwal**, a student of your college for DM Medical Gastroenterology along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
To study the correlation between small intestinal bacterial overgrowth and fecal calprotectin in patients with irritable bowel syndrome

Supervisor
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Co-Supervisor
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The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

S P Singh

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

6. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTORATE OF MEDICINE

IN

GASTROENTEROLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

MARCH, 2020

DR. AAKASH AGGARWAL

DEPARTMENT OF GASTROENTEROLOGY
DAYANAND MEDICAL COLLEGE &HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME
For the degree of	D. M Gastroenterology
Name of the candidate	Dr. Aakash Aggarwal
Supervisor	Dr. Omesh Goyal, Associate Professor, Department of Gastroenterology, DMCH, Ludhiana
Co-supervisor	Dr. Ajit Sood, Professor & Head, Department of Gastroenterology, DMCH, Ludhiana
Institution	Dayanand Medical College & Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits in the absence of any organic cause. The pathophysiology of IBS remains uncertain. Recent studies have considered the role of inflammation, alterations in fecal flora, and bacterial overgrowth in the pathogenesis of IBS, that needs further clarification. Further, because of persistent troublesome symptoms of constipation, diarrhea and abdominal pain, the effect on quality of life, depression and anxiety in IBS patients is also needed to be addressed.

Aim of Study: To study the correlation between small intestinal bacterial overgrowth and fecal calprotectin in patients with irritable bowel syndrome.

Materials & Methods: Prospective and observational study which will be conducted on patients attending the outpatient department or admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India from March 1, 2020 to July 31st 2021 including 1 month Followup.

Clinical Significance: Frequency of small intestinal bacterial overgrowth in IBS, its correlation with fecal calprotectin and psychological assessment of IBS patients.

Keywords: IBS: Irritable Bowel Syndrome, SIBO: Small Intestinal Bacterial Overgrowth.

(Signature) Candidate

(Signature) Co-supervisor

(Signature) Supervisor

(Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME"** exist in the Department of Gastroenterology, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate himself/herself under our supervision and guidance.

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APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Aakash Aggarwal
Department	Gastroenterology
Topic of Thesis	TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME
Likely date of appearing in PG examination	MAY, 2022
Date of enrollment	2nd Sept. 2019
Name of Head of Department	Dr. Ajit Sood
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Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Jaspal Singh (Professor & Head, Surgery)	Mr. A K Jindal (Member, Ethics Committee)
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Approved: Yes / ~~No~~

Dr. Sandeep Puri
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Approved: Yes / ~~No~~

Prof. Arvind Malhotra
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Ethics Committee

Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	Dr. Aakash Aggarwal
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL LUDHIANA
3	COURSE OF STUDY AND SUBJECT	DM GASTROENTEROLOGY
4	DATE OF ADMISSION OF COURSE	2nd Sept 2019
5	TITLE OF THE TOPIC	TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits in the absence of any organic cause. It is the most commonly diagnosed gastrointestinal condition and accounts for approximately 30 percent of all referrals to gastroenterologists.[1] The pathophysiology of IBS remains uncertain.[2] It is viewed as a disorder resulting from an interaction among a number of factors. Despite multiple investigations, data have been conflicting and no abnormality has been found to be specific for this disorder. The traditional focus has been on alterations in gastrointestinal motility and on visceral hypersensitivity. More contemporary studies have considered the role of inflammation, alterations in fecal flora, and bacterial overgrowth.

Small intestinal bacterial overgrowth (SIBO) is associated with an increased number and/or type of bacteria in the upper gastrointestinal (GI) tract. Symptoms of IBS generally resemble SIBO. SIBO has been implicated in pathogenesis of IBS. The concept of a possible association of SIBO with IBS has some logic as several symptoms of these two conditions are similar, and that treatment of SIBO with non-absorbable antibiotics has shown to result in improvement of symptoms in patients with IBS. Various studies are present in literature to assess the association of SIBO with IBS. However, data reporting an association between these two entities have been conflicting, which needs further clarification.

Recent evidence supports a role for inflammation in IBS pathophysiology and generation of IBS symptoms in a subset of patients. Chadwick et al [3] performed studies of colonoscopic biopsy specimens from patients meeting the Rome criteria for clinical diagnosis of IBS. Immunohistological assessment showed an increased number of activated immunocompetent cells, including T-lymphocytes, neutrophils, and mast cells in the intestinal mucosa, suggesting a role for the mucosal immune system in pathogenesis.

Fecal calprotectin is a sensitive marker of intestinal inflammation due to a wide variety of causes, and levels have been shown to correlate with intestinal inflammatory activity.[4]

The use of the calprotectin level as a biomarker of intestinal inflammation could represent an interesting targeted therapeutic approach not only to rule out IBD, but also to identify potential IBS candidates for an anti-inflammatory treatment.

To date, there are only few studies that have assessed the link between fecal calprotectin and SIBO, in IBS patients. Some studies have found positive correlation between SIBO and fecal calprotectin in IBS while others disagree. Therefore, this link needs further clarification for a potential therapeutic alternative in IBS.

Psychological stress is an important factor for the development of irritable bowel syndrome (IBS). More and more clinical and experimental evidence showed that IBS is a combination of irritable bowel and irritable brain.

Alterations in the brain-gut axis are a new concept in IBS pathophysiology. Environmental, cognitive, and emotional states can affect intestinal sensory perception. Corticotropin- releasing hormone (CRH) is a major mediator of stress responses in the brain-gut axis, affecting the functions of both the brain and the gut. Intravenous administration of CRH exacerbated colonic motility, while peripheral administration of a CRH antagonist blocked the stress-induced increase in colonic motility, visceral perception, and negative mood. Several studies have demonstrated brain-gut-interactions using brain imaging. These interactions lead to psychological impact on patients, affecting their mental health and quality of life.

Overall, it seems well established that IBS patients have significantly impaired QoL and show higher levels of psychological distress, specifically of anxiety-related states. Patients of IBS need evaluation in context to stress, anxiety and depression because of persistent and troublesome symptoms of abdominal pain, constipation or diarrhea. The chronic nature of IBS, whose symptoms are often burdening for everyday functioning, indicates that most IBS patients experience

substantial levels of suffering. These psychological manifestations collectively impair quality of life. Because of its chronic nature, IBS has an important impact on QoL, at least equivalent to other chronic gastrointestinal disorders such as gastroesophageal reflux disease or non-digestive diseases such as depression or diabetes. Measuring the impact of irritable bowel syndrome (IBS) on quality of life may provide a method for comparing patients with respect to the severity of the disorder and for judging the efficacy of treatment.

This study is intended to assess the correlation of small intestinal bacterial overgrowth and fecal calprotectin in patients with irritable bowel syndrome, along with symptom severity and quality of life.

6.2 REVIEW OF LITERATURE

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders, characterized by abdominal pain, bloating, and changes in bowel habits. These symptoms cannot be explained by structural abnormalities and there is no specific laboratory test or biomarker for IBS. Therefore, IBS is classified as a functional disorder with diagnosis dependent on the history taking about manifested symptoms and careful physical examination. Although a great deal of research has been carried out in this area, the pathophysiology of IBS is complex and not completely understood.

In recent years, there has been considerable progress in our understanding of the pathophysiology of IBS and small intestinal bacterial overgrowth (SIBO) is emerging as an important causative factor. The diagnosis of SIBO should be suspected in patients with bloating, flatulence, abdominal discomfort, or chronic diarrhea. The diagnosis is established with a positive carbohydrate breath test or a bacterial concentration of $>10^3$ colony forming units/mL in a jejunal aspirate culture. Carbohydrate breath test to diagnose SIBO is preferred as it is simple, non-invasive, and widely available.

Under physiological conditions, glucose is readily absorbed in the small intestine. However if there is bacterial overgrowth in the (upper) small intestine, bacterial fermentation of glucose and production of H_2 can take place prior to the absorption of glucose. So, a breath hydrogen test showing a rise after consuming glucose will mean that there are too many bacteria in the small bowel (i.e. bacterial overgrowth is present).

In support of an association between SIBO and IBS are studies demonstrating abnormal breath hydrogen levels in IBS patients after receiving a test dose of a carbohydrate, as well as improvement in symptoms after eradication of the intestinal bacterial overgrowth.[5] Early studies of SIBO tested only for excess hydrogen in breath following ingestion of sugar, and those studies usually reported diarrhea to be the primary symptom. However, recent studies also test for elevated methane and find that this gas, which is liberated by

anaerobic bacteria, is more often associated with constipation.[6]

Although there are numerous studies supporting SIBO and IBS, there are other studies which have failed to support this association.[7] According to some authors, the abnormal breath tests in patients with IBS may have been due to more rapid orocecal transit in patients with IBS, rather than SIBO.[8] In addition, an improvement of symptoms with antibiotics in patients with IBS may be due to an improvement in intestinal motility or a change in colonic flora rather than SIBO. One study using cultures of jejunal aspirates from 162 patients with IBS and 26 controls found SIBO ($>10^5$ organisms/mL) in 4 percent of both patients and controls. Although a mild increase in bacterial counts was more common in IBS patients (42 versus 12 percent), this was unrelated to small intestinal motility and was not associated with symptoms.[9]

Immunohistological investigation has revealed mucosal immune system activation characterized by alterations in particular immune cells and markers in some patients with irritable bowel syndrome (IBS) (those with diarrhea-predominant IBS and patients with presumed post infectious IBS). Increased numbers of lymphocytes have been reported in the colon and small intestine in patients with IBS. One study in which full-thickness jejunal biopsies were obtained in 10 patients with severe IBS found an increase in lymphocyte infiltration in the myenteric plexus in nine patients and neuron degeneration in six patients. These cells release mediators (nitric oxide, histamine and proteases) capable of stimulating the enteric nervous system, leading to abnormal motor and visceral responses within the intestine. Stool examinations from diarrhea-predominant IBS patients have revealed a high level of serineprotease activity. A fecal extract from these patients, when infused intra-colonically into mice, increased colonic cellular permeability and visceral pain in the mice; these effects were prevented by serine protease inhibitors. The role of intestinal serine-proteases in the pathophysiology of IBS remains under investigation.

Mast cells are effector cells of the immune system. An increased number of mast cells have been demonstrated in the terminal ileum,

jejunum, and colon of IBS patients. Studies have demonstrated a correlation between abdominal pain in IBS and the presence of activated mast cells in proximity to colonic nerves. Cytokines are proteins that are mediators of immune responses. Elevated levels of plasma proinflammatory interleukins have been observed in patients with IBS. In addition, peripheral blood mononuclear cells of IBS patients produce higher amounts of tumor necrosis factor than healthy controls.

Calprotectin is a calcium binding protein accounting for 60% of the cytosolic protein content of neutrophils. Accumulation of neutrophils at the site of inflammation in the gastrointestinal tract results in the release of calprotectin into faeces where it is stable and resistant to bacterial degradation. It identifies patients with IBD (in whom levels may be used to predict impending relapse of disease),[10] patients with NSAID enteropathy, and patients with colorectal carcinoma/adenoma, in all of which there is increased neutrophil recruitment to the inflamed or ulcerated intestinal mucosa.

It has recently been hypothesized that SIBO would represent the trigger for the immune response in IBS: evidence of an activation of the immune response in the intestinal mucosa with an increased number of intraepithelial lymphocytes (in association with increased intestinal permeability) has been reported in patients who develop IBS after recovering from acute infective gastroenteritis.[11]

The potential strength of fecal calprotectin assessment is that it is a direct measure of mucosal inflammatory activity that may be detected at a level insufficient to cause an increase in ESR and CRP, and levels in the stool seem to be unaffected by a variety of non-intestinal conditions, which may result in an elevation of the systemic inflammatory markers.[12]

Many studies were done to assess a link between fecal calprotectin and SIBO in IBS. Melchior et al, were unable to demonstrate such a link between SIBO and elevated calprotectin.[13] Their result was in accordance with Gasbarrini's findings, suggesting that SIBO is not associated with intestinal subclinical inflammatory changes.[14] But in

another study by Marie I et al, it was shown that faecal calprotectin greater than 275 mg/g was predictive of SIBO. But this study was done in a cohort of patients with systemic sclerosis.[15] In IBS patients, David L conducted a study and found a significant relation between SIBO and intestinal inflammation in the form of fecal calprotectin.[16] The role of intestinal microbiota in the pathogenesis of IBS has drawn much attention in recent years.

Recently, disturbance of the bidirectional brain-gut axis is increasingly recognized as a conceptual model of IBS pathophysiology, involving abnormal function in the enteric, autonomic and/or central nervous systems. As stress can result in over activity or under activity along the hypothalamic-pituitary-adrenal (HPA) axis and of the autonomic nervous (ANS), metabolic, and immune systems, it can alter brain-gut interactions, ultimately affecting different physiological functions of the gastrointestinal tract. The relationship between psychological stress and visceral hypersensitivity has been studied and well described by Musial et al[17] and Larauche et al.[18] The co-morbidity of IBS and psychological distress is common, and the prevalence of at least one psychiatric disorder typically ranges from 40% to 60% and has been reported as high as 80%.[19] A strong correlation can also be observed between the severity of IBS and its co-morbid psychiatric disorders, especially depression and anxiety.[20]

Generally, research shows increased levels of anxiety in IBS patients compared to healthy controls. Some IBS patients have psychiatric comorbidities and suffer from anxiety-spectrum disorders; however most of them have heightened but subclinical levels of anxiety-related states. Gastrointestinal-specific anxiety is one such state, which potentially plays a significant role in IBS symptom perpetuation, especially in those patients who are not suffering from anxiety-spectrum disorders. GI-specific anxiety refers to cognitions, emotions, and behaviours related to the digestive system, which are a result of fear and anxiety about GI sensations, symptoms, and the context in which these sensations and symptoms appear. There are findings indicating that GI-specific anxiety is a good predictor of IBS diagnostic

status, symptom severity, and mental QoL in IBS patients. Also, it appears that GI-specific anxiety might be a mediator between trait anxiety and symptom severity.

This study taking multifactorial approach, to assess possible etiology among SIBO and intestinal inflammation, will also be studying psychological aspects of patients in terms of quality of life, symptom severity, depression and anxiety among IBS patients.

6.3 AIMS AND OBJECTIVES OF THE STUDY

AIMS:

Primary aims:

- To study the frequency of small intestinal bacterial overgrowth (SIBO) in patients with irritable bowel syndrome (IBS).
- To study what proportion of IBS patients have evidence of intestinal inflammation (i.e. raised stool calprotectin).
- To study the co-relation between SIBO and fecal calprotectin in IBS patients

Secondary aim:

- To study the co-relation between quality of life and symptoms severity with SIBO and calprotectin in patients with IBS.

7.0 MATERIALS AND METHODS

7.1 SOURCE OF DATA:

This is a Prospective and observational study which will be conducted on patients attending the outpatient department or admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India from March 1, 2020 to July 31st 2021 including 1 month Followup.

7.2 METHOD OF COLLECTION OF DATA

Inclusion criteria – The study will recruit 100 consecutive patients of IBS, visiting Gastroenterology OPD in DMC, Ludhiana, fulfilling the following criteria:

1. Age 18 to 60 years.
2. Fulfilling the diagnostic ROME IV criteria for IBS.

According to the Rome IV criteria, IBS is defined as recurrent abdominal pain, on average, at least one day per week in the last three months, associated with two or more of the following criteria.[21].

- Related to defecation
- Associated with a change in stool frequency
- Associated with a change in stool form (appearance)

Exclusion criteria – Following patients will be excluded from our study:

1. Active use of narcotics, alcohol, opioids or cigarette smoking.
2. Use of probiotics or antibiotics during the last 4 weeks.
3. Age of symptom onset > 50 years.
4. Rectal bleeding or malena.
5. Nocturnal diarrhea.
6. Progressive abdominal pain.
7. Unexplained weight loss.
8. Family history of IBD or colorectal cancer.
9. Laboratory abnormalities indicating an organic cause.
10. Iron deficiency anaemia.

After informed consent, patients will be enrolled in the study. Complete medical history, physical examination, and routine lab investigations

will be noted. Results of the baseline hydrogen breath test (HBT), if done previously will be noted. If baseline HBT results are not available, patients will be advised to get it done, as it is required to rule out small intestinal bacterial overgrowth in IBS. Similarly, patients will be advised to get fecal calprotectin if not done previously to rule out intestinal inflammation. Further, the patients will be classified into two groups, positive and negative, based on the breath test.

Positive Breath Test – Group A

Negative Breath Test – Group B

Both groups will be compared for the association of intestinal inflammation with small intestinal bacterial overgrowth.

The IBS symptom severity scale (IBS-SSS), IBS quality of life scale (IBS-QOL), and psychological questionnaires (HAM-A and HAM-D) will be administered to all patients and compared between both the groups. Characteristics of the IBS patients will be compared between breath test positive and negative group, as well as according to fecal calprotectin level.

Patients will be followed up after 8-12 weeks to assess change in symptom severity and quality of life. Treatment given to the patient by his/her primary physician during this period will be noted.

Questionnaires –

The **Irritable Bowel Severity Scoring System (IBS-SS)** is a validated scale for measuring the overall severity of IBS symptoms. It consists of 5 equally weighted questions about symptoms occurring in the past 10 days: the average intensity of abdominal pain, the number of days with any abdominal pain, the average severity of abdominal distension, dissatisfaction with bowel habits, and the degree to which bowel symptoms interfered with usual activities. Responses to all except the pain frequency question are on a 0-100 numeric scale ("none" to "worst ever"), and the number of days of pain in the past 10 days will be multiplied by 10 to arrive at a 0-100 score for this item. The scores on these 5 questions will be added to arrive a total score of 0-500.[22]

The **Irritable Bowel Syndrome Quality of Life Scale (IBS-QOL)** is a validated, disease specific scale to measure the impact of IBS

symptoms on quality of life. It is a 34-item self report questionnaire with 8 subscales: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction and sexual relationship. Subjects will be asked to answer questions about their feelings over the past 30 days on an ordinal scale (1-"not at all" to 5-"a great deal").[23]

The **HAM-A** was one of the first rating scales developed to measure the severity of anxiety symptoms, and is still widely used today in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Although the HAM-A remains widely used as an outcome measure in clinical trials, it has been criticized for its sometimes poor ability to discriminate between anxiolytic and antidepressant effects, and somatic anxiety versus somatic side effects. The HAM-A does not provide any standardized probe questions. Despite this, the reported levels of inter-rater reliability for the scale appear to be acceptable. [24]

The **HDRS (also known as the Ham-D)** is the most widely used clinician-administered depression assessment scale. The original version contains 17 items (HDRS17) pertaining to symptoms of depression experienced over the past\ week. Although the scale was designed for completion after an unstructured clinical interview, there are now semi-structured interview guides available. The HDRS was originally developed for hospital inpatients, thus the emphasis on melancholic and physical symptoms of depression. A later 21-item version (HDRS21) included 4 items intended to subtype the depression, but which are sometimes, incorrectly, used to rate severity. A limitation of the HDRS is that atypical symptoms of depression (e.g., hypersomnia, hyperphagia) are not assessed.[25]

Breath test –

Breath analysis will be done using GastroCH_4ECK® Gastrolyzer.

Principle - Hydrogen (H2) and Methane (CH4) are generated in the intestinal lumen by bacterial action on carbohydrates in the large or

small intestine. Once the resultant H2 and CH4 are diffused into the bloodstream and then to the alveoli, it can be detected in expiratory air. There exists a correlation between intestinal lumen H2 and CH4 production and H2 and CH4 excretion in expiratory air. Accurate measurement of H2 and CH4 in parts per million (ppm) in expiratory air reveals abnormal breakdown and/or malabsorption of carbohydrates. The GastroCH_4ECK is a simple to operate device with options for both real time breath test and a bag sample test which allows patient breath to be collected in breath bags and analyzed on the device. It also implements a normalizing factor for correcting errors in readings that could have occurred from sample contamination or incorrect breath sample collection. It incorporates single patient use mouthpieces and breath bags for cross contamination control.

Preparation for breath testing –

- No antibiotic, probiotic or bowel preparation (Peglac powder) 2 weeks prior to study.
- No laxatives, fibres or stool softeners, e.g. milk of magnesia, lactulose, isabghol, 1 week prior to study.
- On day before study, milk, juice, soft drink, soda, butter ghee, onion, garlic, cabbage, beans, pickle, pasta shall be avoided.
- Patients will be instructed to skip morning medications, and bring those medications along with them. They will have 12 hours fasting, but sips of water will be allowed.
- On the day of study, chewing gum, smoking, or breath mints shall be avoided.
- Patients will be advised to brush teeth 2 hours prior to study.

Method – It is a simple, non-invasive test. After a short period of fasting i.e. 8-10 hours, a baseline end-expiratory breath sample is collected in breath bag and analyzed. Then, 75 g glucose is mixed with water and given orally. Subsequent breath samples are collected in breath bag and analyzed at every 15 minutes till 1 hour. Then, at the interval of 30 minutes, for the next 1 hour. Total, 6 samples are collected and analyzed over a period of 2 hours.

Interpretation - The glucose/lactulose breath test is diagnostic of SIBO if any one of the following criteria are met [29]:

- An absolute increase in hydrogen by ≥20 ppm above baseline within 90 minutes.
- A methane level ≥10 ppm

Fecal Calprotectin-

Fecal calprotectin will be measured using fluoro-enzyme-immunoassay technique. EliA^{tm} Calprotectin kit will be used on the instrument Phadia 100.

Principle - The EliA^{tm} Calprotectin Wells will be coated with monoclonal antibodies to calprotectin. If present in the patient's specimen, calprotectin will bind to the coated antibodies. After washing away non-bound components, enzyme-labeled antibodies against human calprotectin (EliA^{tm} Calprotectin Conjugate) will be added to form a calprotectin-conjugate complex. After incubation, non-bound conjugate will be washed away and the bound complex will incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture will be measured. The higher the response value, the more calprotectin is present in the specimen. To evaluate test results, the response for patient samples will be compared directly to the response for calibrators.

Method - The procedure will be performed with human stool specimen. Stool extraction and dilution– It will be done using the EliA^{tm} Stool Extraction Kit. The extract can be stored at room temperature for max. 6 h, at 4°C for upto 30 h, and at ≤ -20°C for upto 3 months. Samples must be diluted with EliA^{tm} Sample Diluent. A 1:100 dilution of the samples is required for the EliA^{tm} Calprotectin Test.

Interpretation - Phadia 100 measures calprotectin concentrations in ng/ml. By using a conversion factor given by the lot-specific code of the EliA^{tm} Calprotectin Well, the results are automatically converted to mg/kg. The measuring range (detection limit, upper limit) for EliA^{tm} Calprotectin is from 15.0 to ≥ 3000 mg/kg.

OUTCOME:

- Frequency of small intestinal bacterial overgrowth (SIBO) in patients with irritable bowel syndrome (IBS).
- Proportion of IBS patients that have evidence of intestinal inflammation (i.e. raised stool calprotectin).
- Co-relation between SIBO and fecal calprotectin in patients with IBS.

STATISTICAL ANALYSIS:

Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups will be done using student t- test. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test will be used when the expected frequency is less than 5.A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

NO

7.4 HAS ETHICAL CLEARENCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

ATTACHED HEREWITH

8.0 LIST OF REFERENCES

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PROFORMA

Serial Number

Name

Age/Sex

MRD

Address

Weight Height BMI

Chief complaints:

Total duration of pain-

Frequency of pain per week-

Relation of pain with defecation-

Stool frequency-

Stool form (Bristol score)-

Blood in stools or malena:

Nocturnal diarrhea:

Signature of Candidate Signature of Supervisor

Weight loss:

Personal history :

Alcohol intake

Smoking

Dietary history of FODMAPs :

Medication history (in last 4 weeks):

Prokinetics

Laxatives

Anti-diarrheals

Opioids

Probiotics

Antibiotics

Past history (co-morbidities):

Diabetes Mellitus

Thyroid disorder

Hypertension

CAD

COAD

Any other disorder

Signature of Candidate

Signature of Supervisor

Family history:

IBD

Colorectal carcinoma

Lab parameters:

Hemoglobin

MCV

MCH

TLC

DLC

Platelets

Iron

TIBC

Ferritin

CRP (Quantitative)

ESR

Signature of Candidate

Signature of Supervisor

RFT

Bilirubin (Total/Direct)

ASL/AST

Alkaline phosphatase

Albumin

Fecal Calprotectin

Hydrogen Breath test

IBS-symptom severity score

IBS-QoL score

HAM-A score

HAM-D score

Signature of Candidate

Signature of Supervisor

IBS QUESTIONNAIRE

Name: _____ **G.P. Name:** _____

Address: _____ **Address:** _____

_____ _____

_____ _____

Telephone: _____ **Telephone:** _____

Date of birth: _____

Marital status: Single / Married / Divorced / Widowed / Co-Habit

Occupation: _____ **Sex:** M F

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (ie over the last ten days or so). All information will be kept in **strict** confidence.

1. For questions where a number of different responses are a possibility please circle the response appropriate to you.

2. Some questions will require you to write in an appropriate response.

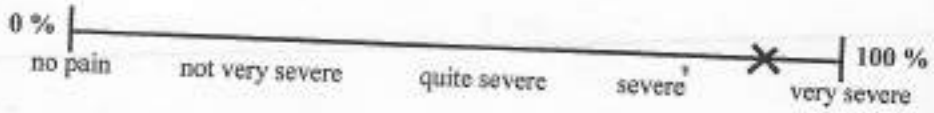
3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example:

How severe was your pain?

Please place your cross (✓) anywhere on the line between 0-100% in order to indicate as accurately as possible the severity of your symptom.

This example shows a severity of approximately 90%



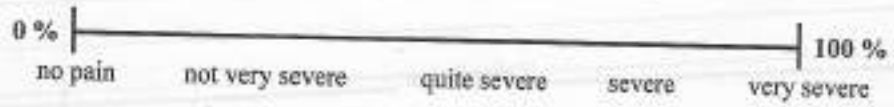
PART 1: SEVERITY SCORE

1. a) ***Do you currently suffer from abdominal (tummy) pain?*** **YES** **NO**
Circle appropriate box

For office use only

b) ***If yes, how severe is your abdominal (tummy) pain?***

SCORE

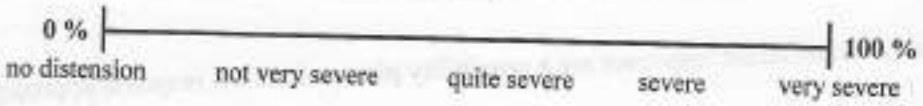


c) ***Please enter the number of days that you get the pain in every ten days.*** *For example if you enter 4 it means that you get pain 4 out of 10 days. If you get pain every day enter 10*

Number of days with pain ☐ x 10 ☐

2. a) ***Do you currently suffer from abdominal distension* (bloating, swollen or tight tummy)*** **YES** **NO**
Circle appropriate box
(women, please ignore distension related to your periods)*

b) ***If yes, how severe is your abdominal distension/tightness***



☐

3. ***How satisfied are you with your bowel habit?***

☐

4. ***Please indicate with a cross on the line below how much your Irritable Bowel Syndrome is affecting or interfering with your life in general.***

☐

IBS SEVERITY SCORE: ☐

PART 2: OTHER IBS DATA

BOWEL HABIT

5. a) ***What is the most number of times you open your bowels per day/week/month?***

Number of times ☐ per day / week / month *(Circle appropriate)*

Note: For some people the answer to part a and b could be the same

b) ***What is the least number of times you open your bowels per day/week/month?***

Number of times ☐ per day / week / month *(Circle appropriate)*

6. In the following questions you may circle more than one answer:

Are your motions ever:

a) ***normal***	often / occasionally / never	*(Circle appropriate)*
b) ***hard***	often / occasionally / never	*(Circle appropriate)*
c) ***very thin (like string)***	often / occasionally / never	*(Circle appropriate)*
d) ***in small pieces (like rabbit pellets)***	often / occasionally / never	*(Circle appropriate)*
e) ***mushy (like porridge)***	often / occasionally / never	*(Circle appropriate)*
f) ***watery***	often / occasionally / never	*(Circle appropriate)*

7. In the following questions you may circle more than one answer:

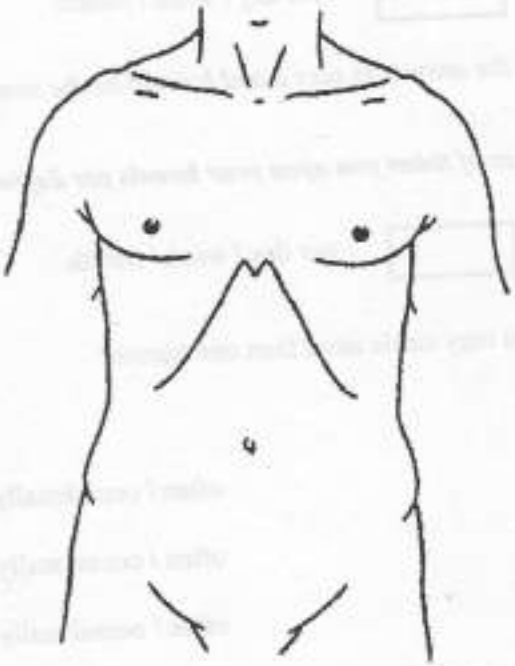
Do you ever:

	Circle appropriate box	
a) ***pass mucus (or slime or jelly) with your motions***	**YES**	**NO**
b) ***pass blood with your motions***	**YES**	**NO**
c) ***have to hurry/rush to the toilet to open your bowels***	**YES**	**NO**
d) ***strain to open your bowels***	**YES**	**NO**
e) ***feel you haven't emptied your bowel completely after you have passed a motion***	**YES**	**NO**

PART 2: Continued

SITE OF PAIN

Please mark with a cross (x) on the diagram below where you get your pain
(use more than one x if necessary)



8. ***Do you ever:***

   a) ***notice your stools are more frequent or loose when you get pain*** **YES** **NO**
   Circle appropriate box

   b) ***notice whether the pain is frequently eased by opening your bowels*** **YES** **NO**
   Circle appropriate box

9. ***In the last year on approximately how many weeks were you:***

   i) ***absent from work due to IBS***
   (enter 52 if you have given up completely work because of IBS) ----------

   ii) ***at work suffering from IBS*** ----------

IBS-Quality of Life Questionairre

About how you feel

Please think about your life over the **past month (last 30 days)**, and look at the statements below. Each statement has five different responses as follow :

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

For each statement, please score your response that best describes your feelings.

Q1. I feel helpless because of my bowel problems.

Q2. I am embarrassed by the smell caused by my bowel problems.

Q3. I am bothered by how much time I spend having a bowel movement.

Q4. I feel I might get other diseases more easily because of my bowel problems.

Q5. I feel fat/bloated because of my bowel problems.

Q6. I feel like I'm losing control of my life because of my bowel problems.

Q7. I feel my life is less enjoyable because of my bowel problems

Q8. I feel uncomfortable when I talk about my bowel problems.

Q9. I feel depressed about my bowel problems.

Q10. I feel isolated from others because of my bowel problems.

Q11. I have to watch the amount of food I eat because of my bowel problems.

Q12. Because of my bowel problems, sexual activity is difficult for me.

Q13. I feel angry that I have bowel problems.

Q14. I feel like I irritate others because of my bowel problems.

Q15. I worry that my bowel problems will get worse.

Q16. I feel irritable because of my bowel problems.

Q17. I worry that people think I exaggerate my bowel problems.

Q18. I feel I get fewer things done because of my bowel problems.

Q19. I have to avoid stressful situations because of my bowel problems.

Q20. My bowel problems reduce my sexual desire.

Q21. My bowel problems limit the kind of clothes I can wear.

Q22. I have to avoid strenuous activity because of my bowel problems.

Q23. I have to watch the kind of food I eat because of my bowel problems.

Q24. Because of my bowel problems, I have difficulty being around people I do not know well.

Q25. I feel sluggish because of my bowel problems.

Q26. I feel unclean because of my bowel problems.

Q27. Long trips are difficult for me because of my bowel problems.

Q28. I feel frustrated that I cannot eat when I want because of my bowel problems.

Q29. It is important to be near a toilet because of my bowel problems.

Q30. My life revolves around my bowel problems.

Q31. I worry about losing control of my bowels

Q32. I fear that I won't be able to have a bowel movement.

Q33. My bowel problems are affecting my closest relationships.

Q34. I feel that no one understands my bowel proble

Hamilton Anxiety Rating Scale (HAM-A)

Below is a list of phrases that describe certain feeling that people have. Rate the patients by finding the answer which best describes the extent to which he/she has these conditions. Select one of the five responses for each of the fourteen questions.

0 = Not present, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very severe.

1 Anxious mood [0] [1] [2] [3] [4]

Worries, anticipation of the worst, fearful anticipation, irritability.

2 Tension [0] [1] [2] [3] [4]

Feelings of tension, fatigability, startle response, moved to tears easily, trembling, feelings of restlessness, inability to relax.

3 Fears [0] [1] [2] [3] [4]

Of dark, of strangers, of being left alone, of animals, of traffic, of crowds.

4 Insomnia [0] [1] [2] [3] [4]

Difficulty in falling asleep, broken sleep, unsatisfying sleep and fatigue on waking, dreams, nightmares, night terrors.

5 Intellectual [0] [1] [2] [3] [4]

Difficulty in concentration, poor memory.

6 Depressed mood [0] [1] [2] [3] [4]

Loss of interest, lack of pleasure in hobbies, depression, early waking, diurnal swing.

7 Somatic (muscular) [0] [1] [2] [3] [4]

Pains and aches, twitching, stiffness, myoclonic jerks, grinding of teeth, unsteady voice, increased muscular tone.

8 Somatic (sensory) [0] [1] [2] [3] [4]

Tinnitus, blurring of vision, hot and cold flushes, feelings of weakness, pricking sensation.

9 Cardiovascular symptoms [0] [1] [2] [3] [4]

Tachycardia, palpitations, pain in chest, throbbing of vessels, fainting feelings, missing beat.

10 Respiratory symptoms [0] [1] [2] [3] [4]

Pressure or constriction in chest, choking feelings, sighing, dyspnea.

11 Gastrointestinal symptoms [0] [1] [2] [3] [4]

Difficulty in swallowing, wind abdominal pain, burning sensations, abdominal fullness, nausea, vomiting, borborygmi, looseness of bowels, loss of weight, constipation.

12 Genitourinary symptoms [0] [1] [2] [3] [4]

Frequency of micturition, urgency of micturition, amenorrhea, menorrhagia, development of frigidity, premature ejaculation, loss of libido, impotence.

13 Autonomic symptoms [0] [1] [2] [3] [4]

Dry mouth, flushing, pallor, tendency to sweat, giddiness, tension headache, raising of hair.

14 Behavior at interview [0] [1] [2] [3] [4]

Fidgeting, restlessness or pacing, tremor of hands, furrowed brow, strained face, sighing or rapid respiration, facial pallor, swallowing, etc.

HAMILTON DEPRESSION RATING SCALE (HAM-D)

(To be administered by a health care professional)

Patient Name _____ **Today's Date** _____

The HAM-D is designed to rate the severity of depression in patients. Although it contains 21 areas, calculate the patient's score on the first 17 answers.

1. **DEPRESSED MOOD**
(Gloomy attitude, pessimism about the future, feeling of sadness, tendency to weep)
0 = Absent
1 = Sadness, etc.
2 = Occasional weeping
3 = Frequent weeping
4 = Extreme symptoms

2. **FEELINGS OF GUILT**
0 = Absent
1 = Self-reproach, feels he/she has let people down
2 = Ideas of guilt
3 = Present illness is a punishment; delusions of guilt
4 = Hallucinations of guilt

3. **SUICIDE**
0 = Absent
1 = Feels life is not worth living
2 = Wishes he/she were dead
3 = Suicidal ideas or gestures
4 = Attempts at suicide

4. **INSOMNIA - Initial**
(Difficulty in falling asleep)
0 = Absent
1 = Occasional
2 = Frequent

5. **INSOMNIA - Middle**
(Complains of being restless and disturbed during the night. Waking during the night.)
0 = Absent
1 = Occasional
2 = Frequent

6. **INSOMNIA - Delayed**
(Waking in early hours of the morning and unable to fall asleep again)
0 = Absent
1 = Occasional
2 = Frequent

7. **WORK AND INTERESTS**
0 = No difficulty
1 = Feelings of incapacity, listlessness, indecision and vacillation
2 = Loss of interest in hobbies, decreased social activities
3 = Productivity decreased
4 = Unable to work. Stopped working because of present illness only. (Absence from work after treatment or recovery may rate a lower score).

8. **RETARDATION**
(Slowness of thought, speech, and activity; apathy; stupor.)
0 = Absent
1 = Slight retardation at interview
2 = Obvious retardation at interview
3 = Interview difficult
4 = Complete stupor

9. **AGITATION**
(Restlessness associated with anxiety.)
0 = Absent
1 = Occasional
2 = Frequent

10. **ANXIETY - PSYCHIC**
0 = No difficulty
1 = Tension and irritability
2 = Worrying about minor matters
3 = Apprehensive attitude
4 = Fears

HAMILTON DEPRESSION RATING SCALE (HAM-D)

(To be administered by a health care professional)

11. ANXIETY - SOMATIC
Gastrointestinal, indigestion
Cardiovascular, palpitation, Headaches
Respiratory, Genito-urinary, etc.
0 = Absent
1 = Mild
2 = Moderate
3 = Severe
4 = Incapacitating

12. SOMATIC SYMPTOMS - GASTROINTESTINAL
(Loss of appetite , heavy feeling in abdomen; constipation)
0 = Absent
1 = Mild
2 = Severe

13. SOMATIC SYMPTOMS - GENERAL
(Heaviness in limbs, back or head; diffuse backache; loss of energy and fatiguability)
0 = Absent
1 = Mild
2 = Severe

14. GENITAL SYMPTOMS
(Loss of libido, menstrual disturbances)
0 = Absent
1 = Mild
2 = Severe

15. HYPOCHONDRIASIS
0 = Not present
1 = Self-absorption (bodily)
2 = Preoccupation with health
3 = Querulous attitude
4 = Hypochondriacal delusions

16. WEIGHT LOSS
0 = No weight loss
1 = Slight
2 = Obvious or severe

17. INSIGHT
(Insight must be interpreted in terms of patient's understanding and background.)
0 = No loss
1 = Partial or doubtfull loss
2 = Loss of insight

TOTAL ITEMS 1 TO 17: _____
0 - 7 = Normal
8 - 13 = Mild Depression
14-18 = Moderate Depression
19 - 22 = Severe Depression
≥ 23 = Very Severe Depression

18. DIURNAL VARIATION
(Symptoms worse in morning or evening. Note which it is.)
0 = No variation
1 = Mild variation; AM ( ) PM ( )
2 = Severe variation; AM ( ) PM ( )

19. DEPERSONALIZATION AND DEREALIZATION
(feelings of unreality, nihilistic ideas)
0 = Absent
1 = Mild
2 = Moderate
3 = Severe
4 = Incapacitating

20. PARANOID SYMPTOMS
(Not with a depressive quality)
0 = None
1 = Suspicious
2 = Ideas of reference
3 = Delusions of reference and persecution
4 = Hallucinations, persecutory

21. OBSESSIONAL SYMPTOMS
(Obsessive thoughts and compulsions against which the patient struggles)
0 = Absent
1 = Mild
2 = Severe

* Adapted from Hamilton, *M.Journal of Neurology, Neurosurgery, and Psychiatry*, 23:56-62, 1960.

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o.....
.....am willing to participate in the study **"TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....
Signature of the Witness

.....
Signature of the patient

Name:

Signature of Candidate

Signature of Supervisor

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य "TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIENTS WITH IRRITABLE BOWEL SYNDROME"में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

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गवाह के हस्ताक्षर

नाम:

_____

रोगी के हस्ताक्षर/अंगूठे का निशान

नाम:

_____

अन्वेषक केहस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ

_____"**TO STUDY THE CORRELATION BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND FECAL CALPROTECTIN IN PATIETNS WITH IRRITABLE BOWEL SYNDROME**" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ